

## METHOD FOR *IN VITRO* SELECTION OF 2'-SUBSTITUTED NUCLEIC ACIDS

### REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to and claims priority to U.S. Provisional Patent Application Serial No. 60/430,761, filed December 3, 2002, U.S. Provisional Patent Application Serial No. 60/487,474, filed July 15, 2003, and U.S. Provisional Patent Application Serial No. 60/517,039, filed November 4, 2003, each of which is herein incorporated by reference in its entirety.

### FIELD OF THE INVENTION

[0002] The invention relates generally to the field of nucleic acids and more particularly to aptamers, and methods for selecting aptamers, incorporating modified nucleotides. The invention further relates to materials and methods for enzymatically producing pools of randomized oligonucleotides having modified nucleotides from which, *e.g.*, aptamers to a specific target can be selected.

### BACKGROUND OF THE INVENTION

[0003] Aptamers are nucleic acid molecules having specific binding affinity to molecules through interactions other than classic Watson-Crick base pairing.

[0004] Aptamers, like peptides generated by phage display or monoclonal antibodies (MAbs), are capable of specifically binding to selected targets and, through binding, block their targets' ability to function. Created by an *in vitro* selection process from pools of random sequence oligonucleotides (Fig. 1), aptamers have been generated for over 100 proteins including growth factors, transcription factors, enzymes, immunoglobulins, and receptors. A typical aptamer is 10-15 kDa in size (30-45 nucleotides), binds its target with sub-nanomolar affinity, and discriminates against closely related targets (*e.g.*, will typically not bind other proteins from the same gene family). A series of structural studies have shown that aptamers are capable of using the same types of binding interactions (hydrogen bonding, electrostatic complementarity, hydrophobic contacts, steric exclusion, *etc*) that drive affinity and specificity in antibody-antigen complexes.

[0005] Aptamers have a number of desirable characteristics for use as therapeutics (and diagnostics) including high specificity and affinity, biological efficacy, and excellent pharmacokinetic properties. In addition, they offer specific competitive advantages over antibodies and other protein biologics, for example:

[0006] 1) Speed and control. Aptamers are produced by an entirely *in vitro* process, allowing for the rapid generation of initial (therapeutic) leads. *In vitro* selection allows the specificity and affinity of the aptamer to be tightly controlled and allows the generation of leads against both toxic and non-immunogenic targets.

[0007] 2) Toxicity and Immunogenicity. Aptamers as a class have demonstrated little or no toxicity or immunogenicity. In chronic dosing of rats or woodchucks with high levels of aptamer (10 mg/kg daily for 90 days), no toxicity is observed by any clinical, cellular, or biochemical measure. Whereas the efficacy of many monoclonal antibodies can be severely limited by immune response to antibodies themselves, it is extremely difficult to elicit antibodies to aptamers (most likely because aptamers cannot be presented by T-cells via the MHC and the immune response is generally trained not to recognize nucleic acid fragments).

[0008] 3) Administration. Whereas all currently approved antibody therapeutics are administered by intravenous infusion (typically over 2-4 hours), aptamers can be administered by subcutaneous injection. This difference is primarily due to the comparatively low solubility and thus large volumes necessary for most therapeutic MAbs. With good solubility (>150 mg/ml) and comparatively low molecular weight (aptamer: 10-50 kDa; antibody: 150 kDa), a weekly dose of aptamer may be delivered by injection in a volume of less than 0.5 ml. Aptamer bioavailability via subcutaneous administration is >80% in monkey studies (Tucker *et al.*, J. Chromatography B. 732: 203-12, 1999). In addition, the small size of aptamers allows them to penetrate into areas of conformational constrictions that do not allow for antibodies or antibody fragments to penetrate, presenting yet another advantage of aptamer-based therapeutics or prophylaxis.

[0009] 4) Scalability and cost. Therapeutic aptamers are chemically synthesized and consequently can be readily scaled as needed to meet production demand. Whereas difficulties in scaling production are currently limiting the availability of some biologics and the capital cost of

a large-scale protein production plant is enormous, a single large-scale synthesizer can produce upwards of 100 kg oligonucleotide per year and requires a relatively modest initial investment. The current cost of goods for aptamer synthesis at the kilogram scale is estimated at \$500/g, comparable to that for highly optimized antibodies. Continuing improvements in process development are expected to lower the cost of goods to < \$100/g in five years.

[0010] 5) Stability. Therapeutic aptamers are chemically robust. They are intrinsically adapted to regain activity following exposure to heat, denaturants, *etc.* and can be stored for extended periods (>1 yr) at room temperature as lyophilized powders. In contrast, antibodies must be stored refrigerated.

[0011] Given the advantages of aptamers as therapeutic agents, it would be beneficial to have materials and methods to prolong or increase the stability of aptamer therapeutics *in vivo*. The present invention provides materials and methods to meet these and other needs.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

[0012] Figure 1 is a schematic representation of the *in vitro* aptamer selection (SELEX™) process from pools of random sequence oligonucleotides.

[0013] Figure 2 shows a 2'-O-methyl (2'-OMe) modified nucleotide, where "B" is a purine or pyrimidine base.

[0014] Figure 3A is a graph of VEGF-binding by three 2'-OMe VEGF aptamers: ARC224, ARC245 and ARC259; Figure 3B shows the sequences and putative secondary structures of these aptamers.

[0015] Figure 4 is a graph of the VEGF-binding by various 2'-OH G variants of ARC224 and ARC225

[0016] Figure 5 is a graph of ARC224 binding to VEGF in HUVEC.

[0017] Figure 6 is a graph of ARC224 binding to VEGF before and after autoclaving, in the presence or absence of EDTA.

[0018] Figures 7A and 7B are graphs of the stability of ARC224 and ARC226, respectively, when incubated at 37 °C in rat plasma.

[0019] Figure 8 is a graph of dRmY SELEX™ Round 6 sequences binding to IgE.

[0020] Figure 9 is a graph of dRmY SELEX™ Round 6 sequences binding to thrombin.

[0021] Figure 10 is a graph of dRmY SELEX™ Round 6 sequences binding to VEGF.

[0022] Figure 11A is a degradation plot of an all 2'-OMe oligonucleotide with 3'-idT, in 95% rat plasma (citrated) at 37 °C, and Figure 11B is a degradation plot of the corresponding dRmY oligonucleotide in 95% rat plasma at 37 °C.

[0023] Figure 12 is a graph of rGmH h-IgE binding clones (Round 6).

[0024] Figure 13A is a graph of round 12 pools for rRmY pool PDGF-BB selection, and Figure 13B is a graph of Round 10 pools for rGmH pool PDGF-BB selection.

[0025] Figure 14 is a graph of dRmY SELEX™ Round 6, 7, 8 and unselected sequences binding to IL-23.

[0026] Figure 15 is a graph of dRmY SELEX™ Round 6, 7 and unselected sequences binding to PDGF-BB.

#### SUMMARY OF THE INVENTION

[0027] The present invention provides materials and methods to produce oligonucleotides of increased stability by transcription under the conditions specified herein which promote the incorporation of modified nucleotides into the oligonucleotide. These modified oligonucleotides can be, for example, aptamers, antisense molecules, RNAi molecules, siRNA molecules, or ribozymes. Preferably, the oligonucleotide is an aptamer.

[0028] In one embodiment, the present invention provides an improved SELEX™ method ("2'-OMe SELEX™") that uses randomized pools of oligonucleotides incorporating modified nucleotides from which aptamers to a specific target can be selected.

[0029] In one embodiment, the present invention provides methods that use modified enzymes to incorporate modified nucleotides into oligonucleotides under a given set of transcription conditions.

[0030] In one embodiment, the present invention provides methods that use a mutated polymerase. In one embodiment, the mutated polymerase is a T7 RNA polymerase. In one

embodiment, a T7 RNA polymerase modified by having a mutation at position 639 (from a tyrosine residue to a phenylalanine residue “Y639F”) and at position 784 (from a histidine residue to an alanine residue “H784A”) is used in various transcription reaction conditions which result in the incorporation of modified nucleotides into the oligonucleotides of the invention.

**[0031]** In another embodiment, a T7 RNA polymerase modified with a mutation at position 639 (from a tyrosine residue to a phenylalanine residue) is used in various transcription reaction conditions which result in the incorporation of modified nucleotides into the oligonucleotides of the invention.

**[0032]** In another embodiment, a T7 RNA polymerase modified with a mutation at position 784 (from a histidine residue to an alanine residue) is used in various transcription reaction conditions which result in the incorporation of modified nucleotides into the aptamers of the invention.

**[0033]** In one embodiment, the present invention provides various transcription reaction mixtures that increase the incorporation of modified nucleotides by the modified enzymes of the invention.

**[0034]** In one embodiment, manganese ions are added to the transcription reaction mixture to increase the incorporation of modified nucleotides by the modified enzymes of the invention.

**[0035]** In another embodiment, 2'-OH GTP is added to the transcription mixture to increase the incorporation of modified nucleotides by the modified enzymes of the invention.

**[0036]** In another embodiment, polyethylene glycol, PEG, is added to the transcription mixture to increase the incorporation of modified nucleotides by the modified enzymes of the invention.

**[0037]** In another embodiment, GMP (or any substituted guanosine) is added to the transcription mixture to increase the incorporation of modified nucleotides by the modified enzymes of the invention.

**[0038]** In one embodiment, a leader sequence incorporated into the 5' end of the fixed region (preferably 20-25 nucleotides in length) at the 5' end of a template oligonucleotide is used to increase the incorporation of modified nucleotides by the modified enzymes of the invention. Preferably, the leader sequence is greater than about 10 nucleotides in length.

**[0039]** In one embodiment, a leader sequence that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0040]** In another embodiment, a leader sequence at least 6 nucleotides long that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0041]** In another embodiment, a leader sequence at least 8 nucleotides long that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0042]** In another embodiment, a leader sequence at least 10 nucleotides long that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0043]** In another embodiment, a leader sequence at least 12 nucleotides long that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0044]** In another embodiment, a leader sequence at least 14 nucleotides long that is composed of up to 100% (inclusive) purine nucleotides is used.

**[0045]** In one embodiment, the present invention provides aptamer therapeutics having modified nucleotides incorporated into their sequence.

**[0046]** In one embodiment, the present invention provides for the use of aptamer therapeutics having modified nucleotides incorporated into their sequence.

**[0047]** In one embodiment, the present invention provides various compositions of nucleotides for transcription for the selection of aptamers with the SELEX<sup>TM</sup> process. In one embodiment, the present invention provides combinations of 2'-OH, 2'-F, 2'-deoxy, and 2'-OMe modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In another embodiment, the present invention provides combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH<sub>2</sub>, and 2'-methoxyethyl modifications of the ATP, GTP, CTP, TTP, and UTP nucleotides. In one embodiment, the present invention provides 5<sup>6</sup> combinations of 2'-OH, 2'-F, 2'-deoxy, 2'-OMe, 2'-NH<sub>2</sub>, and 2'-methoxyethyl modifications the ATP, GTP, CTP, TTP, and UTP nucleotides.

**[0048]** The invention relates to a method for identifying nucleic acid ligands to a target molecule, where the ligands include modified nucleotides, by: a) preparing a transcription reaction mixture comprising a mutated polymerase, one or more 2'-modified nucleotide triphosphates (NTPs), magnesium ions and one or more oligonucleotide transcription templates; b) preparing a

candidate mixture of single-stranded nucleic acids by transcribing the one or more oligonucleotide transcription templates under conditions whereby the mutated polymerase incorporates at least one of the one or more modified nucleotides into each nucleic acid of the candidate mixture, wherein each nucleic acid of the candidate mixture comprises a 2'-modified nucleotide selected from the group consisting of a 2'-position modified pyrimidine and a 2'-position modified purine; c) contacting the candidate mixture with the target molecule; d) partitioning the nucleic acids having an increased affinity to the target molecule relative to the candidate mixture from the remainder of the candidate mixture; and e) amplifying the increased affinity nucleic acids, in vitro, to yield a ligand-enriched mixture of nucleic acids.

**[0049]** The 2'-position modified pyrimidines and 2'-position modified purines include 2'-OH, 2'-deoxy, 2'-O-methyl, 2'-NH<sub>2</sub>, 2'-F, and 2'-methoxy ethyl modifications. Preferably, the 2'-modified nucleotides are 2'-O-methyl or 2'-F nucleotides.

**[0050]** In some embodiments, the mutated polymerase is a mutated T7 RNA polymerase, such as a T7 RNA polymerase having a mutation at position 639 from a tyrosine residue to a phenylalanine residue (Y639F); a T7 RNA polymerase having a mutation at position 784 from a histidine residue to an alanine residue (H784A); a T7 RNA polymerase having a mutation at position 639 from a tyrosine residue to a phenylalanine residue and a mutation at position 784 from a histidine residue to an alanine residue (Y639F/H784A).

**[0051]** In some embodiments, the oligonucleotide transcription template includes a leader sequence incorporated into the 5' end of a fixed region at the 5' end of the oligonucleotide transcription template. The leader sequence, for example, is an all-purine leader sequence. The leader sequence, for example, can be at least 6 nucleotides long; at least 8 nucleotides long; at least 10 nucleotides long; at least 12 nucleotides long; or at least 14 nucleotides long.

**[0052]** In some embodiments, the transcription reaction mixture also includes manganese ions. For example, the concentration of magnesium ions is between 3.0 and 3.5 times greater than the concentration of manganese ions.

**[0053]** In some embodiments of the transcription reaction mixture, each NTP is present at a concentration of 0.5 mM, the concentration of magnesium ions is 5.0 mM, and the concentration of manganese ions is 1.5 mM. In other embodiments of the transcription reaction mixture each NTP is present at a concentration of 1.0 mM, the concentration of magnesium ions is 6.5 mM,

and the concentration of manganese ions is 2.0 mM. In other embodiments of the transcription reaction mixture each NTP is present at a concentration of 2.0 mM, the concentration of magnesium ions is 9.6 mM, and the concentration of manganese ions is 2.9 mM.

**[0054]** In some embodiments, the transcription reaction mixture also includes 2'-OH GTP.

**[0055]** In some embodiments, the transcription reaction mixture also includes a polyalkylene glycol. The polyalkylene glycol can be, *e.g.*, polyethylene glycol (PEG).

**[0056]** In some embodiments, the transcription reaction mixture also includes GMP.

**[0057]** In some embodiments, the method for identifying nucleic acid ligands to a target molecule further includes repeating steps d) partitioning the nucleic acids having an increased affinity to the target molecule relative to the candidate mixture from the remainder of the candidate mixture; and e) amplifying the increased affinity nucleic acids, *in vitro*, to yield a ligand-enriched mixture of nucleic acids.

**[0058]** In some aspects, the invention relates to a nucleic acid ligand to thrombin which was identified according to the method of the invention.

**[0059]** In some aspects, the invention relates to a nucleic acid ligand to vascular endothelial growth factor (VEGF) which was identified according to the method of the invention.

**[0060]** In some aspects, the invention relates to a nucleic acid ligand to IgE which was identified according to the method of the invention.

**[0061]** In some aspects, the invention relates to a nucleic acid ligand to IL-23 which was identified according to the method of the invention.

**[0062]** In some aspects, the invention relates to a nucleic acid ligand to platelet-derived growth factor-BB (PDGF-BB) which was identified according to the method of the invention.

**[0063]** In some embodiments, the transcription reaction mixture includes 2'-OH adenosine triphosphate (ATP), 2'-OH guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).

**[0064]** In some embodiments, the transcription reaction mixture includes 2'-deoxy purine nucleotide triphosphates and 2'-O-methyl pyrimidine nucleotide triphosphates.

**[0065]** In some embodiments, the transcription reaction mixture includes 2'-O-methyl adenosine triphosphate (ATP), 2'-OH guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).



**[0066]** In some embodiments, the transcription reaction mixture includes 2'-O-methyl adenosine triphosphate (ATP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP), 2'-O-methyl guanosine triphosphate (GTP) and deoxy guanosine triphosphate (GTP), wherein the deoxy guanosine triphosphate comprises a maximum of 10% of the total guanosine triphosphate population.

**[0067]** In some embodiments, the transcription reaction mixture includes 2'-O-methyl adenosine triphosphate (ATP), 2'-F guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).

**[0068]** In some embodiments, the transcription reaction mixture includes 2'-deoxy adenosine triphosphate (ATP), 2'-O-methyl guanosine triphosphate (GTP), 2'-O-methyl cytidine triphosphate (CTP) and 2'-O-methyl uridine triphosphate (UTP).

**[0069]** The invention also relates to a method of preparing a nucleic acid comprising one or more modified nucleotides by: preparing a transcription reaction mixture comprising a mutated polymerase, one or more 2'-modified nucleotide triphosphates (NTPs), magnesium ions and one or more oligonucleotide transcription templates; and contacting the one or more oligonucleotide transcription templates with the mutated polymerase under conditions whereby the mutated polymerase incorporates the one or more 2'-modified nucleotides into a nucleic acid transcription product.

**[0070]** 2'-position modified pyrimidines and 2'-position modified purines include 2'-OH, 2'-deoxy, 2'-O-methyl, 2'-NH<sub>2</sub>, 2'-F, and 2'-methoxy ethyl modifications. Preferably, the 2'-modified nucleotides are 2'-O-methyl or 2'-F nucleotides.

**[0071]** In some embodiments, the mutated polymerase is a mutated T7 RNA polymerase, such as a T7 RNA polymerase having a mutation at position 639 from a tyrosine residue to a phenylalanine residue (Y639F); a T7 RNA polymerase having a mutation at position 784 from a histidine residue to an alanine residue (H784A); a T7 RNA polymerase having a mutation at position 639 from a tyrosine residue to a phenylalanine residue and a mutation at position 784 from a histidine residue to an alanine residue (Y639F/H784A).

**[0072]** In some embodiments, the oligonucleotide transcription template includes a leader sequence incorporated into the 5' end of a fixed region at the 5' end of the oligonucleotide transcription template. The leader sequence, for example, is an all-purine leader sequence. The

leader sequence, for example, can be at least 6 nucleotides long; at least 8 nucleotides long; at least 10 nucleotides long; at least 12 nucleotides long; or at least 14 nucleotides long.

[0073] In some embodiments, the transcription reaction mixture also includes manganese ions. For example, the concentration of magnesium ions is between 3.0 and 3.5 times greater than the concentration of manganese ions.

[0074] In some embodiments of the transcription reaction mixture, each NTP is present at a concentration of 0.5 mM, the concentration of magnesium ions is 5.0 mM, and the concentration of manganese ions is 1.5 mM. In other embodiments of the transcription reaction mixture each NTP is present at a concentration of 1.0 mM, the concentration of magnesium ions is 6.5 mM, and the concentration of manganese ions is 2.0 mM. In other embodiments of the transcription reaction mixture each NTP is present at a concentration of 2.0 mM, the concentration of magnesium ions is 9.6 mM, and the concentration of manganese ions is 2.9 mM.

[0075] In some embodiments, the transcription reaction mixture also includes 2'-OH GTP.

[0076] In some embodiments, the transcription reaction mixture also includes a polyalkylene glycol. The polyalkylene glycol can be, *e.g.*, polyethylene glycol (PEG).

[0077] In some embodiments, the transcription reaction mixture also includes GMP.

[0078] The invention also relates to an aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-OH adenosine, substantially all guanosine nucleotides are 2'-OH guanosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, and substantially all uridine nucleotides are 2'-O-methyl uridine. In one embodiment, the aptamer has a sequence composition where at least 80% of all adenosine nucleotides are 2'-OH adenosine, at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In another embodiment, the aptamer has a sequence composition where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In another embodiment, the aptamer has a sequence composition where 100% of all adenosine nucleotides are 2'-OH adenosine, at 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine

nucleotides are 2'-O-methyl cytidine and 100% of all uridine nucleotides are 2'-O-methyl uridine.

**[0079]** The invention also relates to an aptamer composition comprising a sequence where substantially all purine nucleotides are 2'-deoxy purines and substantially all pyrimidine nucleotides are 2'-O-methyl pyrimidines. In one embodiment, the aptamer has a sequence composition where at least 80% of all purine nucleotides are 2'-deoxy purines and at least 80% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines. In another embodiment, the aptamer has a sequence composition where at least 90% of all purine nucleotides are 2'-deoxy purines and at least 90% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines. In another embodiment, the aptamer has a sequence composition where 100% of all purine nucleotides are 2'-deoxy purines and 100% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines.

**[0080]** The invention also relates to an aptamer composition comprising a sequence where substantially all guanosine nucleotides are 2'-OH guanosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all uridine nucleotides are 2'-O-methyl uridine, and substantially all adenosine nucleotides are 2'-O-methyl adenosine. In one embodiment, the aptamer has a sequence composition where at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine. In another embodiment, the aptamer has a sequence composition where at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine. In another embodiment, the aptamer has a sequence composition where 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all uridine nucleotides are 2'-O-methyl uridine, and 100% of all adenosine nucleotides are 2'-O-methyl adenosine.

**[0081]** The invention also relates to an aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-O-methyl adenosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all guanosine nucleotides are 2'-O-methyl

guanosine or deoxy guanosine, substantially all uridine nucleotides are 2'-O-methyl uridine, where less than about 10% of the guanosine nucleotides are deoxy guanosine. In one embodiment, the aptamer has a sequence composition where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine. In another embodiment, the aptamer has a sequence composition where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine. In another embodiment, the aptamer has a sequence composition where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, 100% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine.

**[0082]** The invention also relates to an aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-O-methyl adenosine, substantially all uridine nucleotides are 2'-O-methyl uridine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, and substantially all guanosine nucleotides are 2'-F guanosine sequence. In one embodiment, the aptamer has a sequence composition where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 80% of all guanosine nucleotides are 2'-F guanosine. In another embodiment, the aptamer has a sequence composition where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all guanosine nucleotides are 2'-F guanosine. In another embodiment, the aptamer has a sequence composition where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all uridine nucleotides are 2'-O-methyl

uridine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all guanosine nucleotides are 2'-F guanosine.

**[0083]** The invention also relates to an aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-deoxy adenosine, substantially all cytidine nucleotides are 2'-O-methyl cytidine, substantially all guanosine nucleotides are 2'-O-methyl guanosine, and substantially all uridine nucleotides are 2'-O-methyl uridine. In one embodiment, the aptamer has a sequence composition where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In another embodiment, the aptamer has a sequence composition where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In another embodiment, the aptamer has a sequence composition where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.

**[0084]** The invention also relates to an aptamer composition comprising a sequence where substantially all adenosine nucleotides are 2'-OH adenosine, substantially all guanosine nucleotides are 2'-OH guanosine, substantially all cytidine nucleotides are 2'-OH cytidine, and substantially all uridine nucleotides are 2'-OH uridine. In one embodiment, the aptamer has a sequence composition where at least 80% of all adenosine nucleotides are 2'-OH adenosine, at least 80% of all cytidine nucleotides are 2'-OH cytidine, at least 80% of all guanosine nucleotides are 2'-OH guanosine, and at least 80% of all uridine nucleotides are 2'-OH uridine. In another embodiment, the aptamer has a sequence composition where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all cytidine nucleotides are 2'-OH cytidine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, and at least 90% of all uridine nucleotides are 2'-OH uridine. In another embodiment, the aptamer has a sequence composition where 100% of all adenosine nucleotides are 2'-OH adenosine, 100% of all cytidine nucleotides

are 2'-OH cytidine, 100% of all guanosine nucleotides are 2'-OH guanosine, and 100% of all uridine nucleotides are 2'-OH uridine.

## DETAILED DESCRIPTION OF THE INVENTION

[0085] The details of one or more embodiments of the invention are set forth in the accompanying description below. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. Other features, objects, and advantages of the invention will be apparent from the description. In the specification, the singular forms also include the plural unless the context clearly dictates otherwise. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In the case of conflict, the present Specification will control.

### Modified nucleotide transcription

[0086] The present invention provides materials and methods to produce stabilized oligonucleotides (including, *e.g.*, aptamers) that contain modified nucleotides (*e.g.*, nucleotides which have a modification at the 2' position) which make the oligonucleotide more stable than the unmodified oligonucleotide. The stabilized oligonucleotides produced by the materials and methods of the present invention are also more stable to enzymatic and chemical degradation as well as thermal and physical degradation.

[0087] In order for an aptamer to be suitable for use as a therapeutic, it is preferably inexpensive to synthesize, safe and stable *in vivo*. Wild-type RNA and DNA aptamers are typically not stable *in vivo* because of their susceptibility to degradation by nucleases. Resistance to nuclease degradation can be greatly increased by the incorporation of modifying groups at the 2'-position. Fluoro and amino groups have been successfully incorporated into oligonucleotide libraries from which aptamers have been subsequently selected. However, these modifications greatly increase the cost of synthesis of the resultant aptamer, and may introduce safety concerns because of the possibility that the modified nucleotides could be recycled into host DNA, by degradation of the modified oligonucleotides and subsequent use of the nucleotides as substrates for DNA synthesis.

[0088] Aptamers that contain 2'-O-methyl (2'-OMe) nucleotides overcome many of these drawbacks. Oligonucleotides containing 2'-O-methyl nucleotides are nuclease-resistant and inexpensive to synthesize. Although 2'-O-methyl nucleotides are ubiquitous in biological systems, natural polymerases do not accept 2'-O-methyl NTPs as substrates under physiological conditions, thus there are no safety concerns over the recycling of 2'-O-methyl nucleotides into host DNA. A generic formula for a 2'-OMe nucleotide is shown in Figure 2.

[0089] There are several examples of 2'-OMe-containing aptamers in the literature, see, for example Green et al., *Current Biology* 2, 683-695, 1995. These were generated by the *in vitro* selection of libraries of modified transcripts in which the C and U residues were 2'-fluoro (2'-F) substituted and the A and G residues were 2'-OH. Once functional sequences were identified then each A and G residue was tested for tolerance to 2'-OMe substitution, and the aptamer was re-synthesized having all A and G residues which tolerated 2'-OMe substitution as 2'-OMe residues. Most of the A and G residues of aptamers generated in this two-step fashion tolerate substitution with 2'-OMe residues, although, on average, approximately 20% do not.

Consequently, aptamers generated using this method tend to contain from two to four 2'-OH residues, and stability and cost of synthesis are compromised as a result. By incorporating modified nucleotides into the transcription reaction which generate stabilized oligonucleotides used in oligonucleotide libraries from which aptamers are selected and enriched by SELEX™ (and/or any of its variations and improvements, including those described below), the methods of the current invention eliminate the need for stabilizing the selected aptamer oligonucleotides (*e.g.*, by resynthesizing the aptamer oligonucleotides with modified nucleotides).

[0090] Furthermore, the modified oligonucleotides of the invention can be further stabilized after the selection process has been completed. (See "post-SELEX™ modifications", including truncating, deleting and modification, below.)

### The SELEX™ Method

[0091] A suitable method for generating an aptamer is with the process entitled "Systematic Evolution of Ligands by EXponential enrichment" ("SELEX™") depicted generally in Figure 1. The SELEX™ process is a method for the *in vitro* evolution of nucleic acid molecules with highly specific binding to target molecules and is described in, *e.g.*, U.S. patent application Ser.

No. 07/536,428, filed Jun. 11, 1990, now abandoned, U.S. Pat. No. 5,475,096 entitled "Nucleic Acid Ligands", and U.S. Pat. No. 5,270,163 (see also WO 91/19813) entitled "Nucleic Acid Ligands". Each SELEX™-identified nucleic acid ligand is a specific ligand of a given target compound or molecule. The SELEX™ process is based on the unique insight that nucleic acids have sufficient capacity for forming a variety of two- and three-dimensional structures and sufficient chemical versatility available within their monomers to act as ligands (form specific binding pairs) with virtually any chemical compound, whether monomeric or polymeric. Molecules of any size or composition can serve as targets.

[0092] SELEX™ relies as a starting point upon a large library of single stranded oligonucleotide templates comprising randomized sequences derived from chemical synthesis on a standard DNA synthesizer. In some examples, a population of 100% random oligonucleotides is screened. In others, each oligonucleotide in the population comprises a random sequence and at least one fixed sequence at its 5' and/or 3' end which comprises a sequence shared by all the molecules of the oligonucleotide population. Fixed sequences include sequences such as hybridization sites for PCR primers, promoter sequences for RNA polymerases (*e.g.*, T3, T4, T7, SP6, and the like), restriction sites, or homopolymeric sequences, such as poly A or poly T tracts, catalytic cores, sites for selective binding to affinity columns, and other sequences to facilitate cloning and/or sequencing of an oligonucleotide of interest.

[0093] The random sequence portion of the oligonucleotide can be of any length and can comprise ribonucleotides and/or deoxyribonucleotides and can include modified or non-natural nucleotides or nucleotide analogs. See, *e.g.*, U.S. Patent Nos. 5,958,691; 5,660,985; 5,958,691; 5,698,687; 5,817,635; and 5,672,695, and PCT publication WO 92/07065. Random oligonucleotides can be synthesized from phosphodiester-linked nucleotides using solid phase oligonucleotide synthesis techniques well known in the art (Froehler *et al.*, Nucl. Acid Res. 14:5399-5467 (1986); Froehler *et al.*, Tet. Lett. 27:5575-5578 (1986)). Oligonucleotides can also be synthesized using solution phase methods such as triester synthesis methods (Sood *et al.*, Nucl. Acid Res. 4:2557 (1977); Hirose *et al.*, Tet. Lett., 28:2449 (1978)). Typical syntheses carried out on automated DNA synthesis equipment yield  $10^{15}$ - $10^{17}$  molecules. Sufficiently large regions of random sequence in the sequence design increases the likelihood that each synthesized molecule is likely to represent a unique sequence.



[0094] To synthesize randomized sequences, mixtures of all four nucleotides are added at each nucleotide addition step during the synthesis process, allowing for random incorporation of nucleotides. In one embodiment, random oligonucleotides comprise entirely random sequences; however, in other embodiments, random oligonucleotides can comprise stretches of nonrandom or partially random sequences. Partially random sequences can be created by adding the four nucleotides in different molar ratios at each addition step.

[0095] Template molecules typically contain fixed 5' and 3' terminal sequences which flank an internal region of 30 – 50 random nucleotides. A standard (1  $\mu$ mole) scale synthesis will yield  $10^{15}$  –  $10^{16}$  individual template molecules, sufficient for most SELEX<sup>TM</sup> experiments. The RNA library is generated from this starting library by *in vitro* transcription using recombinant T7 RNA polymerase. This library is then mixed with the target under conditions favorable for binding and subjected to step-wise iterations of binding, partitioning and amplification, using the same general selection scheme, to achieve virtually any desired criterion of binding affinity and selectivity. Starting from a mixture of nucleic acids, preferably comprising a segment of randomized sequence, the SELEX<sup>TM</sup> method includes steps of contacting the mixture with the target under conditions favorable for binding, partitioning unbound nucleic acids from those nucleic acids which have bound specifically to target molecules, dissociating the nucleic acid-target complexes, amplifying the nucleic acids dissociated from the nucleic acid-target complexes to yield a ligand-enriched mixture of nucleic acids, then reiterating the steps of binding, partitioning, dissociating and amplifying through as many cycles as desired to yield highly specific high affinity nucleic acid ligands to the target molecule.

[0096] Within a nucleic acid mixture containing a large number of possible sequences and structures, there is a wide range of binding affinities for a given target. A nucleic acid mixture comprising, for example a 20 nucleotide randomized segment containing only natural unmodified nucleotides can have  $4^{20}$  candidate possibilities. Those which have the higher affinity constants for the target are most likely to bind to the target. After partitioning, dissociation and amplification, a second nucleic acid mixture is generated, enriched for the higher binding affinity candidates. Additional rounds of selection progressively favor the best ligands until the resulting nucleic acid mixture is predominantly composed of only one or a few

sequences. These can then be cloned, sequenced and individually tested for binding affinity as pure ligands.

**[0097]** Cycles of selection and amplification are repeated until a desired goal is achieved. In the most general case, selection/amplification is continued until no significant improvement in binding strength is achieved on repetition of the cycle. The method may be used to sample as many as about  $10^{18}$  different nucleic acid species. The nucleic acids of the test mixture preferably include a randomized sequence portion as well as conserved sequences necessary for efficient amplification. Nucleic acid sequence variants can be produced in a number of ways including synthesis of randomized nucleic acid sequences and size selection from randomly cleaved cellular nucleic acids. The variable sequence portion may contain fully or partially random sequence; it may also contain subportions of conserved sequence incorporated with randomized sequence. Sequence variation in test nucleic acids can be introduced or increased by mutagenesis before or during the selection/amplification iterations.

**[0098]** In one embodiment of SELEX™, the selection process is so efficient at isolating those nucleic acid ligands that bind most strongly to the selected target, that only one cycle of selection and amplification is required. Such an efficient selection may occur, for example, in a chromatographic-type process wherein the ability of nucleic acids to associate with targets bound on a column operates in such a manner that the column is sufficiently able to allow separation and isolation of the highest affinity nucleic acid ligands.

**[0099]** In many cases, it is not necessarily desirable to perform the iterative steps of SELEX™ until a single nucleic acid ligand is identified. The target-specific nucleic acid ligand solution may include a family of nucleic acid structures or motifs that have a number of conserved sequences and a number of sequences which can be substituted or added without significantly affecting the affinity of the nucleic acid ligands to the target. By terminating the SELEX™ process prior to completion, it is possible to determine the sequence of a number of members of the nucleic acid ligand solution family.

**[00100]** A variety of nucleic acid primary, secondary and tertiary structures are known to exist. The structures or motifs that have been shown most commonly to be involved in non-Watson-Crick type interactions are referred to as hairpin loops, symmetric and asymmetric bulges, pseudoknots and myriad combinations of the same. Almost all known cases of such

motifs suggest that they can be formed in a nucleic acid sequence of no more than 30 nucleotides. For this reason, it is often preferred that SELEX™ procedures with contiguous randomized segments be initiated with nucleic acid sequences containing a randomized segment of between about 20-50 nucleotides.

**[00101]** The core SELEX™ method has been modified to achieve a number of specific objectives. For example, U.S. Patent No. 5,707,796 describes the use of SELEX™ in conjunction with gel electrophoresis to select nucleic acid molecules with specific structural characteristics, such as bent DNA. U.S. Patent No. 5,763,177 describes SELEX™ based methods for selecting nucleic acid ligands containing photoreactive groups capable of binding and/or photocrosslinking to and/or photoinactivating a target molecule. U.S. Patent No. 5,567,588 and U.S. Application No. 08/792,075, filed January 31, 1997, entitled “Flow Cell SELEX™”, describe SELEX™ based methods which achieve highly efficient partitioning between oligonucleotides having high and low affinity for a target molecule. U.S. Patent No. 5,496,938 describes methods for obtaining improved nucleic acid ligands after the SELEX™ process has been performed. U.S. Patent No. 5,705,337 describes methods for covalently linking a ligand to its target.

**[00102]** SELEX™ can also be used to obtain nucleic acid ligands that bind to more than one site on the target molecule, and to obtain nucleic acid ligands that include non-nucleic acid species that bind to specific sites on the target. SELEX™ provides means for isolating and identifying nucleic acid ligands which bind to any envisionable target, including large and small biomolecules including proteins (including both nucleic acid-binding proteins and proteins not known to bind nucleic acids as part of their biological function) cofactors and other small molecules. For example, see U.S. Patent No. 5,580,737 which discloses nucleic acid sequences identified through SELEX™ which are capable of binding with high affinity to caffeine and the closely related analog, theophylline.

**[00103]** Counter- SELEX™ is a method for improving the specificity of nucleic acid ligands to a target molecule by eliminating nucleic acid ligand sequences with cross-reactivity to one or more non-target molecules. Counter- SELEX™ is comprised of the steps of a) preparing a candidate mixture of nucleic acids; b) contacting the candidate mixture with the target, wherein nucleic acids having an increased affinity to the target relative to the candidate mixture may be

partitioned from the remainder of the candidate mixture; c) partitioning the increased affinity nucleic acids from the remainder of the candidate mixture; d) contacting the increased affinity nucleic acids with one or more non-target molecules such that nucleic acid ligands with specific affinity for the non-target molecule(s) are removed; and e) amplifying the nucleic acids with specific affinity to the target molecule to yield a mixture of nucleic acids enriched for nucleic acid sequences with a relatively higher affinity and specificity for binding to the target molecule.

**[00104]** One potential problem encountered in the use of nucleic acids as therapeutics and vaccines is that oligonucleotides in their phosphodiester form may be quickly degraded in body fluids by intracellular and/or extracellular enzymes such as endonucleases and exonucleases before the desired effect is manifest. SELEX™ methods therefore encompass the identification of high-affinity nucleic acid ligands which are altered, after selection, to contain modified nucleotides which confer improved characteristics on the ligand, such as improved *in vivo* stability or improved delivery characteristics. Modifications of nucleic acid ligands include, but are not limited to, those which provide other chemical groups that incorporate additional charge, polarizability, hydrophobicity, hydrogen bonding, electrostatic interaction, and fluxionality to the nucleic acid ligand bases or to the nucleic acid ligand as a whole. Modifications include chemical substitutions at the ribose and/or phosphate and/or base positions, such as 2'-position sugar modifications, 5-position pyrimidine modifications, 8-position purine modifications, modifications at exocyclic amines, substitution of 4-thiouridine, substitution of 5-bromo or 5-iodo-uracil; backbone modifications, phosphorothioate or alkyl phosphate modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine and the like. Modifications can also include 3' and 5' modifications such as capping.

**[00105]** In oligonucleotides which comprise modified sugar groups, for example, one or more of the hydroxyl groups is replaced with halogen, aliphatic groups, or functionalized as ethers or amines. Examples of substitution at the 2'-position of the furanose residue include O-alkyl (e.g., O-methyl), O-allyl, S-alkyl, S-allyl, or a halo group. Methods of synthesis of 2'-modified sugars are described in Sproat, *et al.*, Nucl. Acid Res. 19:733-738 (1991); Cotten, *et al.*, Nucl. Acid Res. 19:2629-2635 (1991); and Hobbs, *et al.*, Biochemistry 12:5138-5145 (1973). Other modifications are known to one of ordinary skill in the art.

**[00106]** SELEX™-identified nucleic acid ligands synthesized after selection to contain modified nucleotides are described in U.S. Patent No. 5,660,985, which describes oligonucleotides containing nucleotide derivatives chemically modified at the 5' and 2' positions of pyrimidines. Additionally, U.S. Patent No. 5,756,703 describes oligonucleotides containing various 2'-modified pyrimidines; and U.S. Patent No. 5,580,737 describes highly specific nucleic acid ligands containing one or more nucleotides modified with 2'-amino (2'-NH<sub>2</sub>), 2'-fluoro (2'-F), and/or 2'-O-methyl (2'-OMe) substituents.

**[00107]** The SELEX™ method encompasses combining selected oligonucleotides with other selected oligonucleotides and non-oligonucleotide functional units as described in U.S. Patent No. 5,637,459 and U.S. Patent No. 5,683,867. The SELEX™ method further encompasses combining selected nucleic acid ligands with lipophilic or non-immunogenic high molecular weight compounds in a diagnostic or therapeutic complex, as described in U.S. Patent No. 6,011,020. VEGF nucleic acid ligands that are associated with a lipophilic compound, such as diacyl glycerol or dialkyl glycerol, in a diagnostic or therapeutic complex are described in U.S. Patent No. 5,859,228.

**[00108]** VEGF nucleic acid ligands that are associated with a lipophilic compound, such as a glycerol lipid, or a non-immunogenic high molecular weight compound, such as polyalkylene glycol are further described in U.S. Patent No. 6,051,698. VEGF nucleic acid ligands that are associated with a non-immunogenic, high molecular weight compound or a lipophilic compound are further described in PCT Publication No. WO 98/18480. These patents and applications describe the combination of a broad array of oligonucleotide shapes and other properties, and the efficient amplification and replication properties, of oligonucleotides with the desirable properties of other molecules.

**[00109]** The identification of nucleic acid ligands to small, flexible peptides via the SELEX™ method has also been explored. Small peptides have flexible structures and usually exist in solution in an equilibrium of multiple conformers, and thus it was initially thought that binding affinities may be limited by the conformational entropy lost upon binding a flexible peptide. However, the feasibility of identifying nucleic acid ligands to small peptides in solution was demonstrated in U.S. Patent No. 5,648,214. In this patent, high affinity RNA nucleic acid ligands to substance P, an 11 amino acid peptide, were identified.

[00110] To generate oligonucleotide populations which are resistant to nucleases and hydrolysis, modified oligonucleotides can be used and can include one or more substitute internucleotide linkages, altered sugars, altered bases, or combinations thereof. In one embodiment, oligonucleotides are provided in which the P(O)O group is replaced by P(O)S (“thioate”), P(S)S (“dithioate”), P(O)NR<sub>2</sub> (“amidate”), P(O)R, P(O)OR’, CO or CH<sub>2</sub> (“formacetal”) or 3’-amine (-NH-CH<sub>2</sub>-CH<sub>2</sub>-), wherein each R or R’ is independently H or substituted or unsubstituted alkyl. Linkage groups can be attached to adjacent nucleotide through an -O-, -N-, or -S- linkage. Not all linkages in the oligonucleotide are required to be identical.

[00111] Nucleic acid aptamer molecules are generally selected in a 5 to 20 cycle procedure. In one embodiment, heterogeneity is introduced only in the initial selection stages and does not occur throughout the replicating process.

[00112] The starting library of DNA sequences is generated by automated chemical synthesis on a DNA synthesizer. This library of sequences is transcribed *in vitro* into RNA using T7 RNA polymerase or a modified T7 RNA polymerase, and purified. In one example, the 5’-fixed:random:3’-fixed sequence includes a random sequence having from 30 to 50 nucleotides.

[00113] Incorporation of modified nucleotides into the aptamers of the invention is accomplished before (pre-) the selection process (*e.g.*, a pre-SELEX™ process modification). Optionally, aptamers of the invention in which modified nucleotides have been incorporated by pre-SELEX™ process modification can be further modified by post-SELEX™ process modification (*i.e.*, a post-SELEX™ process modification after a pre-SELEX™ modification). Pre-SELEX™ process modifications yield modified nucleic acid ligands with specificity for the SELEX™ target and also improved *in vivo* stability. Post-SELEX™ process modifications (*e.g.*, modification of previously identified ligands having nucleotides incorporated by pre-SELEX™ process modification) can result in a further improvement of *in vivo* stability without adversely affecting the binding capacity of the nucleic acid ligand having nucleotides incorporated by pre-SELEX™ process modification.

#### Modified Polymerases

[00114] A single mutant T7 polymerase (Y639F) in which the tyrosine residue at position 639 has been changed to phenylalanine readily utilizes 2’deoxy, 2’amino-, and 2’fluoro-

nucleotide triphosphates (NTPs) as substrates and has been widely used to synthesize modified RNAs for a variety of applications. However, this mutant T7 polymerase reportedly can not readily utilize (*e.g.*, incorporate) NTPs with bulkier 2'-substituents, such as 2'-O-methyl (2'-OMe) or 2'-azido (2'-N<sub>3</sub>) substituents. For incorporation of bulky 2' substituents, a double T7 polymerase mutant (Y639F/H784A) having the histidine at position 784 changed to an alanine, or other small amino acid, residue, in addition to the Y639F mutation has been described and has been used to incorporate modified pyrimidine NTPs. A single mutant T7 polymerase (H784A) having the histidine at position 784 changed to an alanine residue has also been described. (Padilla *et al.*, Nucleic Acids Research, 2002, 30: 138). In both the Y639F/H784A double mutant and H784A single mutant T7 polymerases, the change to smaller amino acid residues allows for the incorporation of bulkier nucleotide substrates, *e.g.*, 2'-O methyl substituted nucleotides.

**[00115]** The present invention provides methods and conditions for using these and other modified T7 polymerases having a higher incorporation rate of modified nucleotides having bulky substituents at the furanose 2' position, than wild-type polymerases. Generally, it has been found that under the conditions disclosed herein, the Y693F single mutant can be used for the incorporation of all 2'-OMe substituted NTPs except GTP and the Y639F/H784A double mutant can be used for the incorporation of all 2'-OMe substituted NTPs including GTP. It is expected that the H784A single mutant possesses similar properties when used under the conditions disclosed herein.

**[00116]** The present invention provides methods and conditions for modified T7 polymerases to enzymatically incorporate modified nucleotides into oligonucleotides. Such oligonucleotides may be synthesized entirely of modified nucleotides, or with a subset of modified nucleotides. The modifications can be the same or different. All nucleotides may be modified, and all may contain the same modification. All nucleotides may be modified, but contain different modifications, *e.g.*, all nucleotides containing the same base may have one type of modification, while nucleotides containing other bases may have different types of modification. All purine nucleotides may have one type of modification (or are unmodified), while all pyrimidine nucleotides have another, different type of modification (or are unmodified). In this way, transcripts, or libraries of transcripts are generated using any combination of

modifications, for example, ribonucleotides, (2'-OH, "rN"), deoxyribonucleotides (2'-deoxy), 2'-F, and 2'-OMe nucleotides. A mixture containing 2'-OMe C and U and 2'-OH A and G is called "rRmY"; a mixture containing deoxy A and G and 2'-OMe U and C is called "dRmY"; a mixture containing 2'-OMe A, C, and U, and 2'-OH G is called "rGmH"; a mixture alternately containing 2'-OMe A, C, U and G and 2'-OMe A, U and C and 2'-F G is called "toggle"; a mixture containing 2'-OMe A, U, C, and G, where up to 10% of the G's are deoxy is called "r/mGmH"; a mixture containing 2'-O Me A, U, and C, and 2'-F G is called "fGmH"; and a mixture containing deoxy A, and 2'-OMe C, G and U is called "dAmB".

[00117] A preferred embodiment includes any combination of 2'-OH, 2'-deoxy and 2'-OMe nucleotides. A more preferred embodiment includes any combination of 2'-deoxy and 2'-OMe nucleotides. An even more preferred embodiment is with any combination of 2'-deoxy and 2'-OMe nucleotides in which the pyrimidines are 2'-OMe (such as dRmY, mN or dGmH).

#### 2'-Modified SELEX™

[00118] The present invention provides methods to generate libraries of 2'-modified (*e.g.*, 2'-OMe) RNA transcripts in conditions under which a polymerase accepts 2'-modified NTPs. Preferably, the polymerase is the Y693F/H784A double mutant or the Y693F single mutant. Other polymerases, particularly those that exhibit a high tolerance for bulky 2'-substituents, may also be used in the present invention. Such polymerases can be screened for this capability by assaying their ability to incorporate modified nucleotides under the transcription conditions disclosed herein. A number of factors have been determined to be crucial for the transcription conditions useful in the methods disclosed herein. For example, great increases in the yields of modified transcript are observed when a leader sequence is incorporated into the 5' end of a fixed sequence at the 5' end of the DNA transcription template, such that at least about the first 6 residues of the resultant transcript are all purines.

[00119] Another important factor in obtaining transcripts incorporating modified nucleotides is the presence or concentration of 2'-OH GTP. Transcription can be divided into two phases: the first phase is initiation, during which an NTP is added to the 3'-hydroxyl end of GTP (or another substituted guanosine) to yield a dinucleotide which is then extended by about



10-12 nucleotides, the second phase is elongation, during which transcription proceeds beyond the addition of the first about 10-12 nucleotides. It has been found that small amounts of 2'-OH GTP added to a transcription mixture containing an excess of 2'-OMe GTP are sufficient to enable the polymerase to initiate transcription using 2'-OH GTP, but once transcription enters the elongation phase the reduced discrimination between 2'-OMe and 2'-OH GTP, and the excess of 2'-OMe GTP over 2'-OH GTP allows the incorporation of principally the 2'-OMe GTP.

**[00120]** Another important factor in the incorporation of 2'-OMe into transcripts is the use of both divalent magnesium and manganese in the transcription mixture. Different combinations of concentrations of magnesium chloride and manganese chloride have been found to affect yields of 2'-O-methylated transcripts, the optimum concentration of the magnesium and manganese chloride being dependent on the concentration in the transcription reaction mixture of NTPs which complex divalent metal ions. To obtain the greatest yields of maximally 2' substituted O-methylated transcripts (*i.e.*, all A, C, and U and about 90% of G nucleotides), concentrations of approximately 5 mM magnesium chloride and 1.5 mM manganese chloride are preferred when each NTP is present at a concentration of 0.5 mM. When the concentration of each NTP is 1.0 mM, concentrations of approximately 6.5 mM magnesium chloride and 2.0 mM manganese chloride are preferred. When the concentration of each NTP is 2.0 mM, concentrations of approximately 9.6 mM magnesium chloride and 2.9 mM manganese chloride are preferred. In any case, departures from these concentrations of up to two-fold still give significant amounts of modified transcripts.

**[00121]** Priming transcription with GMP or guanosine is also important. This effect results from the specificity of the polymerase for the initiating nucleotide. As a result, the 5'-terminal nucleotide of any transcript generated in this fashion is likely to be 2'-OH G. The preferred concentration of GMP (or guanosine) is 0.5 mM and even more preferably 1 mM. It has also been found that including PEG, preferably PEG-8000, in the transcription reaction is useful to maximize incorporation of modified nucleotides.

**[00122]** For maximum incorporation of 2'-OMe ATP (100%), UTP(100%), CTP(100%) and GTP (~90%) ("r/mGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01%

(w/v),  $\text{MgCl}_2$  5 mM (6.5 mM where the concentration of each 2'-OMe NTP is 1.0 mM),  $\text{MnCl}_2$  1.5 mM (2.0 mM where the concentration of each 2'-OMe NTP is 1.0 mM), 2'-OMe NTP (each) 500  $\mu\text{M}$  (more preferably, 1.0 mM), 2'-OH GTP 30  $\mu\text{M}$ , 2'-OH GMP 500  $\mu\text{M}$ , pH 7.5, Y639F/H784A T7 RNA Polymerase 15 units/ml, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long. As used herein, one unit of the Y639F/H784A mutant T7 RNA polymerase, or any other mutant T7 RNA polymerase specified herein) is defined as the amount of enzyme required to incorporate 1 nmole of 2'-OMe NTPs into transcripts under the r/mGmH conditions. As used herein, one unit of inorganic pyrophosphatase is defined as the amount of enzyme that will liberate 1.0 mole of inorganic orthophosphate per minute at pH 7.2 and 25 °C.

**[00123]** For maximum incorporation (100%) of 2'-OMe ATP, UTP and CTP ("rGmH") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v),  $\text{MgCl}_2$  5 mM (9.6 mM where the concentration of each 2'-OMe NTP is 2.0 mM),  $\text{MnCl}_2$  1.5 mM (2.9 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500  $\mu\text{M}$  (more preferably, 2.0 mM), pH 7.5, Y639F T7 RNA Polymerase 15 units/ml, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

**[00124]** For maximum incorporation (100%) of 2'-OMe UTP and CTP ("rRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v),  $\text{MgCl}_2$  5 mM (9.6 mM where the concentration of each 2'-OMe NTP is 2.0 mM),  $\text{MnCl}_2$  1.5 mM (2.9 mM where the concentration of each 2'-OMe NTP is 2.0 mM), 2'-OMe NTP (each) 500 $\mu\text{M}$  (more preferably, 2.0 mM), pH 7.5, Y639F/H784A T7 RNA Polymerase 15 units/ml, inorganic pyrophosphatase 5 units/ml, and an all-purine leader sequence of at least 8 nucleotides long.

**[00125]** For maximum incorporation (100%) of deoxy ATP and GTP and 2'-OMe UTP and CTP ("dRmY") into transcripts the following conditions are preferred: HEPES buffer 200 mM, DTT 40 mM, spermidine 2 mM, PEG-8000 10% (w/v), Triton X-100 0.01% (w/v),  $\text{MgCl}_2$  9.6 mM,  $\text{MnCl}_2$  2.9 mM, 2'-OMe NTP (each) 2.0 mM, pH 7.5, Y639F T7 RNA Polymerase 15





methyl cytidine and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-OH adenosine, at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-OH adenosine, 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine and 100% of all uridine nucleotides are 2'-O-methyl uridine.

**[00131]** Under dRmY transcription conditions of the present invention, the transcription reaction mixture comprises 2'-deoxy purine triphosphates and 2'-O-methyl pyrimidine triphosphates. The modified oligonucleotides produced using the dRmY transcription conditions of the present invention comprise substantially all 2'-deoxy purines and 2'-O-methyl pyrimidines. In a preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 80% of all purine nucleotides are 2'-deoxy purines and at least 80% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines. In a more preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where at least 90% of all purine nucleotides are 2'-deoxy purines and at least 90% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all purine nucleotides are 2'-deoxy purines and 100% of all pyrimidine nucleotides are 2'-O-methyl pyrimidines.

**[00132]** Under rGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-OH guanosine triphosphates, 2'-O-methyl cytidine triphosphates, 2'-O-methyl uridine triphosphates, and 2'-O-methyl adenosine triphosphates. The modified oligonucleotides produced using the rGmH transcription mixtures of the present invention comprise substantially all 2'-OH guanosine, 2'-O-methyl cytidine, 2'-O-methyl uridine, and 2'-O-methyl adenosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all guanosine nucleotides are 2'-OH guanosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all uridine nucleotides are 2'-O-

methyl uridine, and at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all guanosine nucleotides are 2'-OH guanosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all guanosine nucleotides are 2'-OH guanosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all uridine nucleotides are 2'-O-methyl uridine, and 100% of all adenosine nucleotides are 2'-O-methyl adenosine.

**[00133]** Under r/mGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-O-methyl adenosine triphosphate, 2'-O-methyl cytidine triphosphate, 2'-O-methyl guanosine triphosphate, 2'-O-methyl uridine triphosphate and deoxy guanosine triphosphate. The resulting modified oligonucleotides produced using the r/mGmH transcription mixtures of the present invention comprise substantially all 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine, wherein the population of guanosine nucleotides has a maximum of about 10% deoxy guanosine. In a preferred embodiment, the resulting r/mGmH modified oligonucleotides of the present invention comprise a sequence where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine. In a most preferred embodiment, the resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine

nucleotides are 2'-O-methyl uridine, and no more than about 10% of all guanosine nucleotides are deoxy guanosine.

**[00134]** Under fGmH transcription conditions of the present invention, the transcription reaction mixture comprises 2'-O-methyl adenosine triphosphates (ATP), 2'-O-methyl uridine triphosphates (UTP), 2'-O-methyl cytidine triphosphates (CTP), and 2'-F guanosine triphosphates. The modified oligonucleotides produced using the fGmH transcription conditions of the present invention comprise substantially all 2'-O-methyl adenosine, 2'-O-methyl uridine, 2'-O-methyl cytidine, and 2'-F guanosine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 80% of all uridine nucleotides are 2'-O-methyl uridine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 80% of all guanosine nucleotides are 2'-F guanosine. In a more preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-O-methyl adenosine, at least 90% of all uridine nucleotides are 2'-O-methyl uridine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, and at least 90% of all guanosine nucleotides are 2'-F guanosine. The resulting modified oligonucleotides comprise a sequence where 100% of all adenosine nucleotides are 2'-O-methyl adenosine, 100% of all uridine nucleotides are 2'-O-methyl uridine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, and 100% of all guanosine nucleotides are 2'-F guanosine.

**[00135]** Under dAmB transcription conditions of the present invention, the transcription reaction mixture comprises 2'-deoxy adenosine triphosphates (dATP), 2'-O-methyl cytidine triphosphates (CTP), 2'-O-methyl guanosine triphosphates (GTP), and 2'-O-methyl uridine triphosphates (UTP). The modified oligonucleotides produced using the dAmB transcription mixtures of the present invention comprise substantially all 2'-deoxy adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine. In a preferred embodiment, the resulting modified oligonucleotides comprise a sequence where at least 80% of all adenosine nucleotides are 2'-deoxy adenosine, at least 80% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 80% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 80% of all uridine nucleotides are 2'-O-methyl uridine. In a more preferred embodiment, the resulting

modified oligonucleotides comprise a sequence where at least 90% of all adenosine nucleotides are 2'-deoxy adenosine, at least 90% of all cytidine nucleotides are 2'-O-methyl cytidine, at least 90% of all guanosine nucleotides are 2'-O-methyl guanosine, and at least 90% of all uridine nucleotides are 2'-O-methyl uridine. In a most preferred embodiment, the resulting modified oligonucleotides of the present invention comprise a sequence where 100% of all adenosine nucleotides are 2'-deoxy adenosine, 100% of all cytidine nucleotides are 2'-O-methyl cytidine, 100% of all guanosine nucleotides are 2'-O-methyl guanosine, and 100% of all uridine nucleotides are 2'-O-methyl uridine.

**[00136]** In each case, the transcription products can then be used as the library in the SELEX™ process to identify aptamers and/or to determine a conserved motif of sequences that have binding specificity to a given target. The resulting sequences are already stabilized, eliminating this step from the process to arrive at a stabilized aptamer sequence and giving a more highly stabilized aptamer as a result. Another advantage of the 2'-OMe SELEX™ process is that the resulting sequences are likely to have fewer 2'-OH nucleotides required in the sequence, possibly none.

**[00137]** As described below, lower but still useful yields of transcripts fully incorporating 2'-OMe substituted nucleotides can be obtained under conditions other than the optimized conditions described above. For example, variations to the above transcription conditions include:

**[00138]** The HEPES buffer concentration can range from 0 to 1 M. The present invention also contemplates the use of other buffering agents having a pKa between 5 and 10, for example without limitation, Tris(hydroxymethyl)aminomethane.

**[00139]** The DTT concentration can range from 0 to 400 mM. The methods of the present invention also provide for the use of other reducing agents, for example without limitation, mercaptoethanol.

**[00140]** The spermidine and/or spermine concentration can range from 0 to 20 mM.

**[00141]** The PEG-8000 concentration can range from 0 to 50 % (w/v). The methods of the present invention also provide for the use of other hydrophilic polymer, for example without limitation, other molecular weight PEG or other polyalkylene glycols.



[00142] The Triton X-100 concentration can range from 0 to 0.1% (w/v). The methods of the present invention also provide for the use of other non-ionic detergents, for example without limitation, other detergents, including other Triton-X detergents.

[00143] The  $\text{MgCl}_2$  concentration can range from 0.5 mM to 50 mM. The  $\text{MnCl}_2$  concentration can range from 0.15 mM to 15 mM. Both  $\text{MgCl}_2$  and  $\text{MnCl}_2$  must be present within the ranges described and in a preferred embodiment are present in about a 10 to about 3 ratio of  $\text{MgCl}_2$ : $\text{MnCl}_2$ , preferably, the ratio is about 3-5, more preferably, the ratio is about 3 to about 4.

[00144] The 2'-OMe NTP concentration (each NTP) can range from 5  $\mu\text{M}$  to 5 mM.

[00145] The 2'-OH GTP concentration can range from 0  $\mu\text{M}$  to 300  $\mu\text{M}$ .

[00146] The 2'-OH GMP concentration can range from 0 to 5 mM.

[00147] The pH can range from pH 6 to pH 9. The methods of the present invention can be practiced within the pH range of activity of most polymerases that incorporate modified nucleotides.

[00148] In addition, the methods of the present invention provide for the optional use of chelating agents in the transcription reaction condition, for example without limitation, EDTA, EGTA, and DTT.

#### Pharmaceutical Compositions

[00149] The invention also includes pharmaceutical compositions containing the aptamer molecules described herein. In some embodiments, the compositions are suitable for internal use and include an effective amount of a pharmacologically active compound of the invention, alone or in combination, with one or more pharmaceutically acceptable carriers. The compounds are especially useful in that they have very low, if any toxicity.

[00150] Compositions of the invention can be used to treat or prevent a pathology, such as a disease or disorder, or alleviate the symptoms of such disease or disorder in a patient. Compositions of the invention are useful for administration to a subject suffering from, or predisposed to, a disease or disorder which is related to or derived from a target to which the

aptamers specifically bind.

**[00151]** For example, the target is a protein involved with a pathology, for example, the target protein causes the pathology.

**[00152]** Compositions of the invention can be used in a method for treating a patient having a pathology. The method involves administering to the patient a composition comprising aptamers that bind a target (*e.g.*, a protein) involved with the pathology, so that binding of the composition to the target alters the biological function of the target, thereby treating the pathology.

**[00153]** The patient having a pathology, *e.g.* the patient treated by the methods of this invention can be a mammal, or more particularly, a human.

**[00154]** In practice, the compounds or their pharmaceutically acceptable salts, are administered in amounts which will be sufficient to exert their desired biological activity.

**[00155]** For instance, for oral administration in the form of a tablet or capsule (*e.g.*, a gelatin capsule), the active drug component can be combined with an oral, non-toxic pharmaceutically acceptable inert carrier such as ethanol, glycerol, water and the like. Moreover, when desired or necessary, suitable binders, lubricants, disintegrating agents and coloring agents can also be incorporated into the mixture. Suitable binders include starch, magnesium aluminum silicate, starch paste, gelatin, methylcellulose, sodium carboxymethylcellulose and/or polyvinylpyrrolidone, natural sugars such as glucose or beta-lactose, corn sweeteners, natural and synthetic gums such as acacia, tragacanth or sodium alginate, polyethylene glycol, waxes and the like. Lubricants used in these dosage forms include sodium oleate, sodium stearate, magnesium stearate, sodium benzoate, sodium acetate, sodium chloride, silica, talcum, stearic acid, its magnesium or calcium salt and/or polyethyleneglycol and the like. Disintegrators include, without limitation, starch, methyl cellulose, agar, bentonite, xanthan gum starches, agar, alginic acid or its sodium salt, or effervescent mixtures, and the like. Diluents, include, *e.g.*, lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and/or glycine.

**[00156]** Injectable compositions are preferably aqueous isotonic solutions or suspensions, and suppositories are advantageously prepared from fatty emulsions or suspensions. The compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or

buffers. In addition, they may also contain other therapeutically valuable substances. The compositions are prepared according to conventional mixing, granulating or coating methods, respectively, and contain about 0.1 to 75%, preferably about 1 to 50%, of the active ingredient.

**[00157]** The compounds of the invention can also be administered in such oral dosage forms as timed release and sustained release tablets or capsules, pills, powders, granules, elixirs, tinctures, suspensions, syrups and emulsions.

**[00158]** Liquid, particularly injectable compositions can, for example, be prepared by dissolving, dispersing, *etc.* The active compound is dissolved in or mixed with a pharmaceutically pure solvent such as, for example, water, saline, aqueous dextrose, glycerol, ethanol, and the like, to thereby form the injectable solution or suspension. Additionally, solid forms suitable for dissolving in liquid prior to injection can be formulated. Injectable compositions are preferably aqueous isotonic solutions or suspensions. The compositions may be sterilized and/or contain adjuvants, such as preserving, stabilizing, wetting or emulsifying agents, solution promoters, salts for regulating the osmotic pressure and/or buffers. In addition, they may also contain other therapeutically valuable substances.

**[00159]** The compounds of the present invention can be administered in intravenous (both bolus and infusion), intraperitoneal, subcutaneous or intramuscular form, all using forms well known to those of ordinary skill in the pharmaceutical arts. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions.

**[00160]** Parental injectable administration is generally used for subcutaneous, intramuscular or intravenous injections and infusions. Additionally, one approach for parenteral administration employs the implantation of a slow-release or sustained-released systems, which assures that a constant level of dosage is maintained, according to U.S. Pat. No. 3,710,795, incorporated herein by reference.

**[00161]** Furthermore, preferred compounds for the present invention can be administered in intranasal form *via* topical use of suitable intranasal vehicles, or via transdermal routes, using those forms of transdermal skin patches well known to those of ordinary skill in that art. To be administered in the form of a transdermal delivery system, the dosage administration will, of course, be continuous rather than intermittent throughout the dosage regimen. Other preferred topical preparations include creams, ointments, lotions, aerosol sprays and gels, wherein the

concentration of active ingredient would range from 0.01% to 15%, w/w or w/v.

**[00162]** For solid compositions, excipients include pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound defined above, may be also formulated as suppositories using for example, polyalkylene glycols, for example, propylene glycol, as the carrier. In some embodiments, suppositories are advantageously prepared from fatty emulsions or suspensions.

**[00163]** The compounds of the present invention can also be administered in the form of liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, containing cholesterol, stearylamine or phosphatidylcholines. In some embodiments, a film of lipid components is hydrated with an aqueous solution of drug to a form lipid layer encapsulating the drug, as described in U.S. Pat. No. 5,262,564. For example, the aptamer molecules described herein can be provided as a complex with a lipophilic compound or non-immunogenic, high molecular weight compound constructed using methods known in the art. An example of nucleic-acid associated complexes is provided in US Patent No. 6,011,020.

**[00164]** The compounds of the present invention may also be coupled with soluble polymers as targetable drug carriers. Such polymers can include polyvinylpyrrolidone, pyran copolymer, polyhydroxypropyl-methacrylamide-phenol, polyhydroxyethylaspanamidephenol, or polyethyleneoxidepolylysine substituted with palmitoyl residues. Furthermore, the compounds of the present invention may be coupled to a class of biodegradable polymers useful in achieving controlled release of a drug, for example, polylactic acid, polyepsilon caprolactone, polyhydroxy butyric acid, polyorthoesters, polyacetals, polydihydropyrans, polycyanoacrylates and cross-linked or amphipathic block copolymers of hydrogels.

**[00165]** If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents, and other substances such as for example, sodium acetate, triethanolamine, oleate, *etc.*

**[00166]** The dosage regimen utilizing the compounds is selected in accordance with a variety of factors including type, species, age, weight, sex and medical condition of the patient; the severity of the condition to be treated; the route of administration; the renal and hepatic function of the

patient; and the particular compound or salt thereof employed. An ordinarily skilled physician or veterinarian can readily determine and prescribe the effective amount of the drug required to prevent, counter or arrest the progress of the condition.

[00167] Oral dosages of the present invention, when used for the indicated effects, will range between about 0.05 to 1000 mg/day orally. The compositions are preferably provided in the form of scored tablets containing 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 25.0, 50.0, 100.0, 250.0, 500.0 and 1000.0 mg of active ingredient. Effective plasma levels of the compounds of the present invention range from 0.002 mg to 50 mg per kg of body weight per day.

[00168] Compounds of the present invention may be administered in a single daily dose, or the total daily dosage may be administered in divided doses of two, three or four times daily.

[00169] All publications and patent documents cited herein are incorporated herein by reference as if each such publication or document was specifically and individually indicated to be incorporated herein by reference. Citation of publications and patent documents is not intended as an admission that any is pertinent prior art, nor does it constitute any admission as to the contents or date of the same.

[00170] The invention having now been described by way of written description, those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples below are for purposes of illustration and not limitation of the claims that follow.

## EXAMPLES

### EXAMPLE 1          2'-OMe SELEX™ Against Thrombin and VEGF targets

[00171] A library of approximately  $3 \times 10^{14}$  unique transcription templates, each containing a random region of thirty contiguous nucleotides, was synthesized as described below, and PCR amplified. Cloning and sequencing of this library demonstrated that the composition of the random region in this library was approximately 25% of each nucleotide. The DNA library was purified away from unincorporated dNTPs by gel-filtration and ethanol-precipitation. Modified transcripts were then generated from a mixture containing 500 uM of each of the four 2'-OMe NTPs, *i.e.*, A, C, U and G, and 30 uM 2'-OH GTP ("r/mGmH"). In addition, modified transcripts were generated from mixtures containing part modified nucleotides and part

ribonucleotides or all ribonucleotides namely, a mixture containing all 2'-OH nucleotides (rN); a mixture containing 2'-OMe C and U and 2'-OH A and G (rRmY); a mixture containing 2'-OMe A, C, and U, and 2'-OH G ("rGmH"); and a mixture alternately containing 2'-OMe A, C, U and G and 2'-OMe A, U and C and 2'-F G ("toggle"). These modified transcripts were then used in SELEX™ against targets – *e.g.*, VEGF and thrombin.

[00172] Generally, after gel-purification and DNase-treatment these modified transcripts were dissolved in PBS for VEGF or 1X ASB (150 mM KCl, 20 mM HEPES, 10 mM MgCl<sub>2</sub>, 1 mM DTT, 0.05 % Tween20, pH 7.4) for thrombin, and incubated for one hour in an empty well on a hydrophobic multiwell plate to subtract plastic-binding sequences. The supernatant was then transferred to a well that had previously been incubated for one hour at room temperature in PBS for VEGF or in ASBND (150 mM KCl, 20 mM HEPES, 10 mM MgCl<sub>2</sub>, 1 mM DTT, pH 7.4) for thrombin. After a one hour incubation the well was washed and bound sequences were reverse-transcribed *in situ* using thermoscript reverse transcriptase (Invitrogen) at 65 °C for one hour. The resultant cDNA was then PCR-amplified, separated from dNTPs by gel-filtration, and used to generate modified transcripts for input into the next round of selection. After 10 rounds of selection and amplification the ability of the resultant library to bind to VEGF or thrombin was assessed by Dot-Blot. At this point, the library was cloned, sequenced and individual clones were assayed for their ability to bind VEGF or thrombin. Using this combination of sequence and clonal binding data, sequence motifs were identified.

[00173] One VEGF aptamer motif, exemplified by ARC224, which was common to both the r/mGmH and toggle selections, was used to design smaller synthetic constructs which were also assayed for binding to VEGF and ultimately minimized aptamers to VEGF were identified, ARC245 and ARC259, both of which are 23 nucleotides long. Another VEGF aptamer motif, exemplified by ARC226, which was common to all 2'-OMe selections, was also identified. The ARC224 aptamer produced by the methods of the present invention has the sequence 5'-mCmGmAmUmAmUmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmAmUmUmCmG-3T (SEQ ID No. 184) where "m" represents a 2'-O-methyl substitution.

5-mGmAmUmCmAmUmGmCmAmUGmUmGmGmAmUmCmGmCmGmGmAmUmC-[3T]-  
3' (SEQ ID No. 186).

5'-mAmUmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmAmU-[3T]-3'  
(SEQ ID No. 187).

5'-mAmCmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmGmCmGMu-[3T]-3'  
(SEQ ID No. 188).

**[00177]** Figure 3A is a graph of VEGF binding by ARC224, ARC245 and ARC259. A schematic representation of the secondary structure of these aptamers is presented in Figure 3B.

**[00178]** All residues in ARC224, ARC226 and ARC245 are 2'-OMe and all constructs (initially identified by SELEX™) were generated by solid-phase chemical synthesis. The K<sub>D</sub> values of these aptamers, determined by dot-blot in PBS, are as follows: ARC224 3.9 nM, ARC245 2.1 nM, ARC259 1.4 nM.

**[00179] Reagents.** All reagents were acquired from Sigma (St. Louis, MO) except where otherwise stated.

**[00180] Oligonucleotide synthesis.** DNA syntheses were undertaken according to standard protocols using an Expedite 8909 DNA synthesizer (Applied Biosystems, Foster City, CA). The DNA library used in this study had the following sequence: ARC254:

5'-CATCGATGCTAGTCGTAAACGATCCNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  
NCGAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:1) in which each N has an equal probability of being each of the four nucleotides. 2'-OMe RNA syntheses, including those containing 2'-OH nucleotides, were undertaken according to standard protocols using a 3900 DNA Synthesizer (Applied Biosystems, Foster City, CA). All oligonucleotides were purified by denaturing PAGE except PCR and RT primers.

**[00181] 2'-OMe Library Generation.** The synthetic DNA library (1.5 nmol) was amplified by PCR under standard conditions with the following primers: 3'-primer

5'-CATCGATGCTAGTCGTAACGATCC-3' (SEQ ID NO:454) and 5'-primer 5'-TAATACGACTCACTATAGGGAGAGGAGAGAAACGTTCTCG-3' (SEQ ID NO:455). The resultant library of double-stranded transcription templates was precipitated and separated from unincorporated nucleotides by gel-filtration. At no point was the library denatured, either by thermal means or by exposure to low-salt conditions. r/mGmH transcription was performed under the following conditions to produce template for the first round of selection: double-stranded DNA template 200 nM, HEPES 200 mM, DTT 40 mM, Triton X-100 0.01%, Spermidine 2 mM, 2'-O-methyl ATP, CTP, GTP and UTP 500  $\mu$ M each, 2'-OH GTP 30  $\mu$ M, GMP 500  $\mu$ M, MgCl<sub>2</sub> 5.0 mM, MnCl<sub>2</sub> 1.5 mM, inorganic pyrophosphatase 0.5 units per 100  $\mu$ L reaction, Y639F/H784A T7 RNA polymerase 1.5 units per 100  $\mu$ L reaction pH 7.5 and 10% w/v PEG and were incubated at 37 °C overnight. The resultant transcripts were purified by denaturing 10% PAGE, eluted from the gel, incubated with RQ1 DNase (Promega, Madison WI), phenol-extracted, chloroform-extracted, precipitated and taken up in PBS. For the initiation of selection transcripts were additionally generated by the direct chemical synthesis of 2'-OMe RNA, these were purified by denaturing 10% polyacrylamide gel electrophoresis, eluted from the gel and taken up in PBS.

**[00182]** For the rN, rRmY and rGmH transcriptions, the transcription conditions were as follows, where 1X Tc buffer is: 200 mM HEPES, 40 mM DTT, 2 mM Spermidine, 0.01% Triton X-100, pH 7.5.

**[00183]** When 2'-OH A, C, U and G (rN) conditions were used, the transcription reaction conditions were MgCl<sub>2</sub> 25 mM, each NTP 5 mM, 1X Tc buffer, 10% w/v PEG, T7 RNA polymerase 1.5 units, and 50-200 nM double stranded template (200 nM of template was used in Round 1 to increase diversity and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction using conditions described herein, was used).

**[00184]** When 2'-OMe C and U and 2'-OH A and G (rRmY) conditions were used, the transcription reaction conditions were 1X Tc buffer, 50-200 nM double stranded template (200 nM of template was used in Round 1 to increase diversity and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction using conditions described herein, was used), 5.0 mM MgCl<sub>2</sub>, 1.5 mM MnCl<sub>2</sub>, 0.5 mM each base, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F/H784A T7 RNA polymerase.



[00185] When 2'-OMe A, C, and U and 2'-OH G (rGmH) conditions were used, the transcription reaction conditions were 1X Tc buffer, 50-200 nM double stranded DNA template (200 nM of template was used in Round 1 to increase diversity for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction using conditions described herein, was used), 5.0 mM MgCl<sub>2</sub>, 1.5 mM MnCl<sub>2</sub>, 0.5 mM each base, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F single mutant T7 RNA polymerase in 100 µl volume.

[00186] When 2'-OMe A, C, U and 2'-F G conditions were used, the transcription reaction conditions were as for rGmH, except 0.5 mM 2'-F GTP is used instead of 2'-OH GTP.

[00187] **Reverse Transcription.** The reverse transcription conditions used during SELEX™ are as follows (100 µL reaction volume): 1X Thermo buffer (Invitrogen), 4 µM primer, 10 mM DTT, 0.2 mM each dNTP, 200 µM Vanadate nucleotide inhibitor, 10 µg/ml tRNA, Thermoscript RT enzyme 1.5 units (Invitrogen). Reverse transcriptase reaction yields are lower for 2'-OMe templates. PCR reaction conditions are as follows 1X ThermoPol buffer (NEB), 0.5 µM 5' primer, 0.5 µM 3' primer 0.2 mM each dNTP, Taq DNA Polymerase 5 units (NEB).

[00188] **2'-OMe SELEX™ Protocol.** As noted above, SELEX™ was performed with the modified transcripts against each of two targets (VEGF and Thrombin) using 5 kinds of transcripts for a total of 10 selections. The five kinds of transcripts were: "rN" (all 2'-OH), "rRmY" (2'-OH A, G, 2'-OMe C, U), "rGmH" (2'-OH G, 2'-OMe C, U, A), "r/mGmH" (2'-OMe A, U, G, C 500 uM, 2'-OH G 30 uM), "toggle" (alternately "r/mGmH" and 2'-OMe A, U, C, 2'-F G).

[00189] All of the selections directed against VEGF generated VEGF specific aptamers while only the rN and rRmY selections against thrombin generated thrombin specific aptamers. The aptamer sequences identified in these selections are set forth in Tables 1 through 5 (VEGF) and Tables 6 through 10 (thrombin) below.

[00190] The sequences are from SELEX™ round 11 except for Thrombin "rGmH", "r/mGmH" and "toggle" which are from round 5, VEGF "r/mGmH" which is from round 10 and VEGF "toggle" which is from round 8.

[00191] The selection was performed by initially immobilizing the protein by hydrophobic absorption to "NUNC MAXY" plates, washing away the protein that didn't bind, incubating the

library of 2'-OMe-substituted transcripts with the immobilized protein, washing away the transcripts that didn't bind, performing RT directly in the plate, then PCR, and then transcribing the resultant double-stranded DNA template under the appropriate transcription conditions.

[00192] Binding assays were performed with trace  $^{32}\text{P}$ -body-labelled transcripts that were incubated with various protein concentrations in silanized wells, these were then passed through a sandwich of a nitrocellulose membrane over a nylon membrane. Protein-bound RNA is visualized on the NC membrane, unbound RNA on the nylon membrane. The proportion binding is then used to calculate affinity (see Figures 4, 5, and 6). For example, the binding characteristics of various 2'-OH G variants of ARC224 (all 2-OMe) are shown in Figure 4. The nomenclature "mGXG" indicates a substitution of 2'-OH G for 2'-OMe G at position "X", as numbered sequentially from the 5'-terminus. Thus, mG7G ARC224 is ARC224 with a 2'-OH at position 7. ARC225 is ARC224 with 2'-OMe to 2'-OH substitutions at positions 7, 10, 14, 16, 19, 22 and 24. All constructs (initially identified by SELEX<sup>TM</sup>) were generated by solid-phase chemical synthesis. These data were generated by dot-blot in PBS. The fully 2'-OMe aptamer, ARC224, has superior VEGF-binding characteristics when compared to any of the 2'-OH substituted variants studied.

[00193] Figure 5 is a plot of ARC224 and ARC225 binding to VEGF. This graph indicates that ARC224 binds VEGF in a manner which inhibits the biological function of VEGF.  $^{125}\text{I}$ -labeled VEGF was incubated with the aptamer and this mixture was then incubated with human umbilical cord vascular endothelial cells (HUVEC). The supernatant was removed, the cells were washed, and bound VEGF was counted in a scintillation counter. ARC225 has the same sequence as ARC224 and 2'-OMe to 2'-OH substitutions at positions 7, 10, 14, 16, 19, 22 and 24 numbered from the 5'-terminus. These data indicate that the  $\text{IC}_{50}$  of ARC224 is approximately 2 nM.

[00194] Figure 6 is a binding curve plot of ARC224 binding to VEGF before and after autoclaving, with or without EDTA. Figure 6 shows both the proportion of aptamer that is functional and the  $\text{IC}_{50}$  for binding to VEGF before and after autoclaving for 25 minutes with a peak temperature of 125 °C. These data were determined by the inhibition by unlabeled ARC224 of the binding of  $^{32}\text{P}$ -labeled ARC224 to 1 nM VEGF in PBS as measured by dot-blot in PBS. Where indicated, samples contained 1 mM EDTA. All constructs (initially identified by

SELEX™) were generated by solid-phase chemical synthesis. No degradation of ARC224 was observed within the limitations of this assay.

[00195] Degradation studies show that incubation in plasma at 37 °C over 4 days induces so little degradation that measuring a half-life is not possible, but is at least in excess of 4 days (see, *e.g.*, Figure 7). Figures 7A and 7B are plots of the stability of ARC224 and ARC226, respectively, when incubated at 37 °C in rat plasma. As indicated in the figure, both ARC224 and ACR226 showed no detectable degradation after for 4 days in rat plasma. In these experiments, 5'-labeled ARC224 and ARC226 were incubated in rat plasma at 37 °C and analyzed by denaturing PAGE. All constructs (initially identified by SELEX™) were generated by solid-phase chemical synthesis. The half-life appears to be in excess of 100 hours.

[00196] Tables 1 through Table 10 below show the DNA sequences of aptamers corresponding to the transcribed aptamers isolated from the various libraries, *i.e.* rN, rRmY, rGmH, and r/mGmH, as indicated. The sequence of the aptamers will have uridine residues instead of thymidine residues in the DNA sequences shown. Table 11 shows the stabilized aptamer sequences obtained by the methods of the present invention. As used herein, "3T" refers to an inverted thymidine nucleotide attached to the oligonucleotide phosphodiester backbone at the 5' position, the resulting oligo having two 5'-OH ends and is thus resistant to 3' nucleases.

[00197] Unless noted otherwise, individual sequences listed in the various tables represent the cDNA clones of the aptamers that were selected under the SELEX conditions provided. The actual aptamers provided in the invention are those corresponding sequences comprising the rN, mN, rRmY, rGmH, r/mGmH, dRmY and toggle combinations of residues, as indicated in the text.

#### 2'-OMe SELEX™ Results.

[00198] TABLE 1 – Corresponding cDNAs of the VEGF Aptamer Sequences – all 2'-OH (rN)

SEQ ID No. 3 >PB.97.126.F\_43-H1  
GGGAGAGGAGAGAACGTTCTCGAAATGATGCATGTTCTGTAATGGCAGTATTGGATCGTTACAACCTAGCATCGATG

SEQ ID No. 4 >PB.97.126.F\_43-A2  
GGGAGAGGAGAGAACGTTCTCGTGCCGAGGTCCGGAACCTTGATGATTGGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 5 >PB.97.126.F\_48-A1  
GGGAGAGGAGAGAACGTTCTCGCATTTGGGCTAGTTGTGAAATGGCAGTATTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 6 >PB.97.126.F\_48-B1

GGGAGAGGAGAGAACGTTCTCGAATCGTAGATAGTCGTGAAATGGCAGTATTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 7 >PB.97.126.F\_48-C1  
GGGAGAGGAGAGAACGTTCTCGTTCTAGTCGGTACGATATGTTGACGAATCCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 8 >PB.97.126.F\_48-D1  
GGGAGAGGAGAGAACGTTCTCGTTTGATGAGGCGGACATAATCCGTGCCGAGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 9 >PB.97.126.F\_48-E1  
GGGAGAGGAGAGAACGTTCTCGAAGGAAAAGAGTTTAGTATTGGCCGTCCGTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 10 >PB.97.126.F\_48-F1  
GGGAGAGGAGAGAACGTTCTCGTGCCGAGGTCCGGAACCTTGATGATTGGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 11 >PB.97.126.F\_48-G1  
GGGAGAGGAGAGAACGTTCTCGTACGGTCCATTGAGTTTGAGATGTCGCCATGGATCGTTACGACTAGCATCGATG

SEQ ID No. 12 >PB.97.126.F\_48-B2  
GGGAGAGGAGAGAACGTTCTCGAGTTAGTGGTAAGTATGTTGAATTGTCCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 13 >PB.97.126.F\_48-C2  
GGGAGAGGAGAGAACGTTCTCGCACGGATGGCGAGAACAGAGATTGCTAGGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 14 >PB.97.126.F\_48-D2  
GGGAGAGGAGAGAACGTTCTCGNTANCGNTNCGCCNTGCTAACGCNTANTTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 15 >PB.97.126.F\_48-E2  
GGGAGAGGAGAGAACGTTCTCGAAGATGAGTTTTGTGCTGAAATGGCAGTATTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 16 >PB.97.126.F\_48-F2  
GGGAGAGGAGAGAACGTTCTCGGGATGCCGGATTGATTTCTGATGGGTACTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 17 >PB.97.126.F\_48-G2  
GGGAGAGGAGAGAACGTTCTCGAATGGAATGCATGTCCATCGCTAGCATTTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 18 >PB.97.126.F\_48-H2  
GGGAGAGGAGAGAACGTTCTCGTGCTGAGGTCCGGAACCTTGATGATTGGCGGGATCGTTNCTAGCATCGATG

SEQ ID No. 19 >PB.97.126.F\_48-A3  
GGGAGAGGAGAGAACGTTCTCGCTAATTGCTGAGTCGTGAAGTGGCAGTATTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 20 >PB.97.126.F\_48-B3  
GGGAGAGGAGAGAACGTTCTCGTAACGATGTCCGGGGCGAAAGGCTAGCATGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 21 >PB.97.126.F\_48-C3  
GGGAGAGGAGAGAACGTTCTCGATGCGATTGTGAGATTTGTAAGATAGCTGTGGATCGTTACGACTAGCATCGATG

**[00199] TABLE 2 – Corresponding cDNAs of the VEGF Aptamer Sequences – 2'-OH AG, 2'-OMe CU (rRmY)**

SEQ ID No. 22 >PB.97.126.G\_43-D3  
GGGAGAGGAGAGAACGTTCTCGCAGAAAACATCTTTGCGGTTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 23 >PB.97.126.G\_43-G3  
GGGAGAGGAGAGAACGTTCTCGAAAAAGANANCNNCTTCNGAATACATGCCGATCGTTACGACTAGCATCGATG

SEQ ID No. 24 >PB.97.126.G\_48-E3  
GGGAGAGGAGAGAACGTTCTCGAGAGTGATTTCGATGCTTCANGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 25 >PB.97.126.G\_48-F3  
GGGAGAGGAGAGAACGTTCTCGACANNNTNGCTNGGTTGANTACATGTGNNTNTCNNNANCNNTNNTCTNTNANAG  
GGG

SEQ ID No. 26 >PB.97.126.G\_48-H3  
GGGAGAGGAGAGAACGTTCTCGAAGAAGGAAAGCTGCAAGTCGAATACACGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 27 >PB.97.126.G\_48-A4  
GGGAGAGGAGAGAACGTTCTCGCAAAAACATCGATTACAGTTGAGTACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 28 >PB.97.126.G\_48-B4  
GGGAGAGGAGAGAACGTTCTCGAGACATCATTGCTCGTTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 29 >PB.97.126.G\_48-C4  
GGGAGAGGAGAGAACGTTCTCGCCAAAGTAGCTTCGACAGTCGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 30 >PB.97.126.G\_48-D4  
GGGAGAGGAGAGAACGTTCTCGAAAATCAGTACTGTGCAGTCGAATACATGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 31 >PB.97.126.G\_48-E4  
GGGAGAGGAGAGAACGTTCTCGTAATGACATCAATGCTTCTTGAATACAGGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 32 >PB.97.126.G\_48-F4  
GGGAGAGGAGAGAACGTTCTCGAGAAAACGATCTGTGACGTGTAATCCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 33 >PB.97.126.G\_48-G4  
GGGAGAGGAGAGAACGTTCTCGCAACAAACGTCGACGCTTCTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 34 >PB.97.126.G\_48-H4  
GGGAGAGGAGAGAACGTTCTCGTGATCATAGAAATGCTAGCTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 35 >PB.97.126.G\_48-A5  
GGGAGAGGAGAGAACGTTCTCGCAGCGTAAATGCTTTTTCGAAGTACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 36 SEQ ID No. >PB.97.126.G\_48-B5  
GGGAGAGGAGAGAACGTTCTCGCCAAGAATCAATCGCTTGTCGAATACATGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 37 >PB.97.126.G\_48-C5  
GGGAGAGGAGAGAACGTTCTCGTGATCATAGAAATGCTAGCTGAGTACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 38 >PB.97.126.G\_48-D5  
GGGAGAGGAGAGAACGTTCTCGCAGAAAACATCTTTGCGGTTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 39 >PB.97.126.G\_48-E5  
GGGAGAGGAGAGAACGTTCTCGNAAACANNCATCTATTGNAGTTGAATACATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 40 >PB.97.126.G\_48-F5  
GGGAGAGGAGAGAACGTTCTCGCTAAAGATTGCTGCTTGCCGAATACATGTGGATCGTTACGACTAGCATCGATG

**[00200] TABLE 3 – Corresponding cDNAs of the VEGF Aptamer Sequences – 2'-OH G, 2'-OMe CUA (rGmH)**

SEQ ID No. 41 >PB.97.126.H\_43-H6  
GGGAGAGGAGAGAACGTTCTCGGGTTTTGTCTGCGTTTTGTGCGTTGAACCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 42 >PB.97.126.H\_43-F7  
GGGAGAGGAGAGAACGTTCTCGTGATTACGTGATGAGGATCCGCGTTTTCTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 43 >PB.97.126.H\_43-H7  
GGGAGAGGAGAGAACGTTCTCGTTAGTGAAACGATCATGCATGTGGATCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 44 >PB.97.126.H\_48-H5  
GGGAGAGGAGAGAACGTTCTCGTGTTCAATTCGTTTGCTTATCGTTGCATGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 45 >PB.97.126.H\_48-A6  
AGGAGAGGAGAGAACGTTCTCGGCAGAGTGTGATGTGCATCCGCACGTGCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 46 >PB.97.126.H\_48-B6  
GGGAGAGGAGAGAACGTTCTCGTTAGTAAATACGATCGTGCATGTGGATCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 47 >PB.97.126.H\_48-C6  
GGGAGAGGAGAGAACGCCCCCTGATTNCGTGAAGAGGATCCGCANTTTTCNCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 48 >PB.97.126.H\_48-D6  
GGGAGAGGAGAGAACGTTCTCGTGGCTTTGGAACGGGTACGGATTTGGCACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 49 >PB.97.126.H\_48-E6  
GGGAGAGGAGAGAACGTTCTCGTGATTACGTGATGAGGATCCGCGTTTTCTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 50 >PB.97.126.H\_48-F6  
GGGAGAGGAGAGAACGTTCTCGTCATTGGTGACNGCGTTGCATGTGGATCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 51 >PB.97.126.H\_48-G6  
GGGAGAGGAGAGAACGTTCTCGNTGGTNNAANGCTTTTGTNNGGNTANNTGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 52 SEQ ID No. >PB.97.126.H\_48-A7  
GGGAGAGGAGAGAACGTTCTCGTGGCTTTGGAACGAATTCGGATTTGGCACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 53 >PB.97.126.H\_48-B7  
GGGAGAGGAGAGAACGTTCTCGTGCGATGTCGTGGATTTCCGTTTCGCAAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 54 >PB.97.126.H\_48-C7  
GGGAGAGGAGAGAACGTTCTCGTGAAGCAGATGTCGTTGGCGACTTAGAGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 55 >PB.97.126.H\_48-D7  
GGGAGAGGAGAGAACGTTCTCGTGATTTCTGTGATGAGGATCCGCGTTTTCTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 56 >PB.97.126.H\_48-E7  
GGGAGAGGAGAGAACGTTCTCGCTAGTAACGATGACTTGATGAGCATCCGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 57 >PB.97.126.H\_48-G7  
GGGAGAGGAGAGAACGTTCTCGTCATAAGTAACGACGTTGCATGTGGATCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 58 >PB.97.126.H\_48-A8  
GGGAGAGGAGAGAACGTTCTCGCAAGGAGATGGTTGCTAGCTGAGTACATGTGGATCGTTACGACTAGCATCGATG

**[00201]** TABLE 4 – Corresponding cDNAs of the VEGF Aptamer Sequences – 2'-OMe AUGC (r/mGmH, each G has a 90% probability of having a 2'-OMe group incorporated therein)

SEQ ID No. 59 PB.97.126.I\_43-B8  
GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTCGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 60 >PB.97.126.I\_48-C8  
GGGAGAGGAGAGAACGTTCTCGTGCGACGGGCTTCTTGTGTCATTTCGCATGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 61 >PB.97.126.I\_48-D8  
GGGAGAGGAGAGAACGTTCTCGGCATTGCAGTTGATAGGTCGCGCAGTGCTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 62 >PB.97.126.I\_48-E8  
GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTCTGAGAAGTCGCGCATTTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 63 >PB.97.126.I\_48-F8  
GGGAGAGGAGAGAACGTTCTCGTGTAGCAAGCATGTGGATCGCGACTGCACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 64 >PB.97.126.I\_48-G8  
GGGAGAGGAGAGAACGTTCTCGGATAAGCAGTTGAGATGTCGCGCTTTGACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 65 >PB.97.126.I\_48-H8  
GGGAGAGGAGAGAACGTTCTCGATGANCANTTTGAGAAGTCGCGCTTGTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 66 >PB.97.126.I\_48-A9  
GGGAGAGGAGAGAACGTTCTCGAGTAATGCAGTGGAAGTCGCGCATTACCTGGGATCGTTACGACTAGCATCATG

SEQ ID No. 67 >PB.97.126.I\_48-B9  
GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTTCGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 68 >PB.97.126.I\_48-C9  
GGGAGAGGAGAGAACGTTCTCGTGATNCAGTTGANAAGTCNCGCATACAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 69 >PB.97.126.I\_48-D9  
GGGAGAGGAGAGAACGTTCTCGAGTAATGCTGTGGAAGTCGCGCATTTCCTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 70 >PB.97.126.I\_48-D8  
GGGAGAGGAGAGAACGTTCTCGGCATTGCAGTTGATAGGTGCGCGAGTGCTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 71 >PB.97.126.I\_48-F9  
GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGGAAGTCGCGCATTTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 72 >PB.97.126.I\_48-G9  
GGGAGAGGAGAGAACGTTCTCGCNATATGCTGTTTGANAANTCGCGCATTTCGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 73 >PB.97.126.I\_48-H9  
GGGAGAGGAGAGAACGTTCTCGCGTAGATTGGGCTGAATGGGATATCTTTAGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 74 >PB.97.126.I\_48-B10  
GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCTTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 75 >PB.97.126.I\_48-D10  
GGGAGAGGAGAGAACGTTCTCGTCAATCTGATGTAGCCTCACGTGGGCGGAGTCGGATCGTTACGACTAGCATCGATG

**[00202] TABLE 5 – Corresponding cDNAs of the VEGF Aptamer Sequences – alternately “r/mGmH” and 2’-OMe AUC, 2’-F G (toggle)**

SEQ ID No. 76 >PB.97.126.J\_48-F10  
GGGAGAGGAGAGAACGTTCTCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 77 >PB.97.126.J\_48-G10  
GGGAGAGGAGAGAACGTTCTCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 78 >PB.97.126.J\_48-H10  
GGGAGAGGAGAGAACGTTCTCGGTGGTGTGCTGAACTGTCGCGTTTCGCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 79 >PB.97.126.J\_48-A11  
GGGAGAGGAGAGAACGTTCTCGTCGCGATTGCATATTTCCGCCTTGCTGTGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 80 >PB.97.126.J\_48-B11

GGGAGAGGAGAGAACGTTCTCGCGATTTGCAGTTTGAGATGTCGCGCATTTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 81 >PB.97.126.J\_48-C11

GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTTCGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 82 >PB.97.126.J\_48-D11

GGGAGAGGAGAGAACGTTCTCGTTGGTGCAGTTTGAGATGTCGCGCACCTTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 83 >PB.97.126.J\_48-E11

GGGAGAGGAGAGAACGTTCTCGGTATTGGTTCCATTAAAGCTGGACACTCTGCTCCGGGATCGTTACGACTAGCATCGA  
TG

SEQ ID No. 84 >PB.97.126.J\_48-F11

GGGAGAGGAGAGAACGTTCTCGTTGGTGCAGTTTGAGATGTCGCGCGCCTTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 85 >PB.97.126.J\_48-G11

GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTTCGAGGGATCGTTACNACTAGCATCGATG

SEQ ID No. 86 >PB.97.126.J\_48-A12

GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTTCGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 87 >PB.97.126.J\_48-B12

GGGAGAGGAGAGAACGCTCTCGGGGACNNAANNCGAATTGNCGCGTGNGTCCGGGGGAGCGCCCGACTAGTCATCGA  
TG

SEQ ID No. 88 >PB.97.126.J\_48-C12

GGGAGAGGAGAGAACGTTCTCGCGATATGNANTTTGAGAAGTCGCGCATTTCGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 89 >PB.97.126.J\_48-D12

GGGAGAGGAGAGAACGTTCTCGGTGTACAGCTTGAGATGTCGCGTACTCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 90 >PB.97.126.J\_48-E12

GGGAGAGGAGAGAACGTTCTCGCGATATGCAGTTTGAGAAGTCGCGCATTTCGGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 91 >PB.97.126.J\_48-F12

GGGAGAGGAGAGAACGTTCTCGAGTAAGAAAGCTGAATGGTCGCACTTCTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 92 >PB.97.126.J\_48-G12

AGGGAGAGGAAGAACGTTCTCGCGATGTGCAGTTTGAGAAGTCGCGCATTTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 93 >PB.97.126.J\_48-H12

GGGAGAGGAGAGAACGTTCTCGAAAGAATCAGCATGCGGATCGCGGCTTTTCGGGATCGTTACGACTAGCATCGATG

### [00203] TABLE 6 – Corresponding cDNAs of the Thrombin Aptamer Sequences – all 2'-OH (rN)

SEQ ID No. 94 >PB.97.126.A\_44-A1

GGGAGAGGAGAGAACGTTCTCGANTCCANTNTNCNTGGAGGAGTAAGTACCTGAGGGATCGTTACGACTAGCATCGAT  
G

SEQ ID No. 95 >PB.97.126.A\_44-B1

GGGAGAGGAGAGAACGTTCTCGGGAAACAAGGAACCTTAGAGTTANTTGACCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 96 >PB.97.126.A\_44-C1

GGGAGAGGAGAGAACGTTCTCGTACCATGCAAGGAACATAATAGTTAGCGTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 97 >PB.97.126.A\_44-D1

GGGAGAGGAGAGAACGTTCTCGGGACACAAGGAACACAATAGTTAGTGTACGGGATCGTTACGACTAGCATCGATG



SEQ ID No. 98 >PB.97.126.A\_44-E1  
GGGAGAGGAGAGAACGTTCTCGTCTGCAAGGAACACAATAGTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 99 >PB.97.126.A\_44-F1  
GGGAGAGGAGAGAACGTTCTCGCGCCAACAAGCTGGAGTACTTAGAGCGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 100 >PB.97.126.A\_44-G1  
GGGAGAGGAGAGAACGTTCTCGATTGCAAAATAGCTGTAGAACTAAGCAATCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 101 >PB.97.126.A\_44-H1  
GGGAGAGGAGAGAACGTTCTCGTGAGATGACTATGTTAAGATGACGCTGTTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 102 >PB.97.126.A\_44-A2  
GGGAGAGGAGAGAACGTTCTCGGGANACAAGGAACNCAATATTTAGTGAACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 103 >PB.97.126.A\_44-B2  
GGGAGAGGAGAGAACGTTCTCGCCAAGGAACACAATAGTTAGGTGAGAATCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 104 >PB.97.126.A\_44-C2  
GGGAGAGGAGAGAACGTTCTCGGTACAAGGAACACAATAGTTAGTGCCGTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 105 >PB.97.126.A\_44-D2  
GGGAGAGGAGAGAACGTTCTCGATTCAACGGTCCAAAAAGCTGTAGTACTTAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 106 >PB.97.126.A\_44-E2  
GGGAGAGGAGAGAACGTTCTCGCAATGCAAGGAACACAATAGTTAGCAGCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 107 >PB.97.126.A\_44-F2  
GGGAGAGGAGAGAACGTTCTCGAAAGGAGAAAGCTGAAGTACTTACTATGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 108 >PB.97.126.A\_44-G2  
GGGAGAGGAGAGAACGTTCTCGCACAAAGGAACACAATAGTTAGTGCAAGACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 109 >PB.97.126.A\_44-A3  
GGGAGAGGAGAGAACGTTCTCGCACAAAGGAACACTACGAGTTAGTGTGGGAGTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 110 >PB.97.126.A\_44-B3  
GGGAGAGGAGAGAACGTTCTCGCACAAAGGAACACAATAGTTAGTGCAAGACGGGATCGTTACGACTAGCATCGATA

SEQ ID No. 111 >PB.97.126.A\_44-C3  
GGGAGAGGAGAGAACGTTCTCGGCGGGAAAATAGCTGTAGTACTAACCACGGATCGTTACGACTAGCATCGATG

**[00204] TABLE 7 – Corresponding cDNAs of the Thrombin Aptamer Sequences – 2'-OH AG, 2'-OMe CU (rRmY)**

SEQ ID No. 112 >PB.97.126.B\_44-E3  
GGGAGAGGAGAGAACGTTCTCGGCCTCAAGGAAAAGAAAATTTAGAGGCCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 113 >PB.97.126.B\_44-F3  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 114 >PB.97.126.B\_44-G3  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 115 >PB.97.126.B\_44-H3  
GGGAGAGGAGAGAACGTTCTCGGAGCCAAGGAAACGAAGATTTAGGCTCATTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 116 >PB.97.126.B\_44-A4  
GGGAGAGGAGAGAACGTTCTCGATCACAAGAAATGTGGGANGGTAGTGATNCNNNTCGTTNCGACTAGCATCGATG

SEQ ID No. 117 >PB.97.126.B\_44-B4  
GGGAGAGGAGAGAACGTTCTCGTCGAAAGGGAGCTTTGTCTCGGGACAGAACGGATCGTTACGACTAGCATCGATG

SEQ ID No. 118 >PB.97.126.B\_44-C4  
GGGAGAGGAGAGAACGNTCTCGTGCAAAGATAGCTGGAGGACTAATGCGGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 119 >PB.97.126.B\_44-D4  
GGGAGAGGAGAGAACGTTCTCGTCGAAAGGGAGCTTTGTCTCGGGACAGAACGGATCGTTACGACTAGCATCGATG

SEQ ID No. 120 >PB.97.126.B\_44-E4  
GGGAGAGGAGAGAACGTTCTCGNCNAAGGNGAGCTTTGTCCCNGGACANAANGNATCGTTACAACACTAGCATCGATG

SEQ ID No. 121 >PB.97.126.B\_44-F4  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 122 >PB.97.126.B\_44-G4  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 123 >PB.97.126.B\_44-H4  
GGGAGAGGAGAGAACGTTCTCGGCGCAAAAAAGCTGGAGTACTTAGTGTCGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 124 >PB.97.126.B\_44-A5  
GGGAGAGGAGAGAACGTTCTCGTCGAAAGGGAGCTTTGTCTCGGGACAGAACGGATCGTTACGACTAGCATCGATG

SEQ ID No. 125 >PB.97.126.B\_44-B5  
GGGAGAGGAGAGAACGTTCTCGACACAAGAAAGCTGCAGAACTTAGGGTCGTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 126 >PB.97.126.B\_44-C5  
GGGAGAGGAGAGAACGTTCTCGGAACNGGATTGTTGAAGGACTAANTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 127 >PB.97.126.B\_44-D5  
GGGAGAGGAGAGAACGTTCTCGGCCTCAAGGGAAAGAAAATTTAGAGGCCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 128 >PB.97.126.B\_44-E5  
GGGAGAGGAGAGAACGTTCTCGGAACAAGCTTAGAAATTCGCACCCTTGCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 129 >PB.97.126.B\_44-F5  
GGGAGAGGAGAGAACGTTCTCGAAAGAAAAGCTGGAGAACTTACTTCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 130 >PB.97.126.B\_44-G5  
GGGAGAGGAGAGAACGTTCTCGGTGATTGTACTCACATAGAAATGGCAACACTGGGATCGTTACGACTAGCATCGATG

**[00205] TABLE 8 – Corresponding cDNAs of the Thrombin Aptamer Sequences – 2'-OH G, 2'-OMe CUA (rGmH)**

SEQ ID No. 131 >PB.97.126.C\_44-H5  
GGGAGAGGAGAGAACGTTCTCGGGTTCAAGGAACATGATAGTTAGAACCCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 132 >PB.97.126.C\_44-A6  
GGGAGAGGAGAGAACGTTCTCGTTCCGAAAGGAACACAATAGTTATCGGATTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 133 >PB.97.126.C\_44-B6  
GGGAGAGGAGAGAACGTTCTCGTCTGCAAGGAACACAATAGTTAGCATTGCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 134 >PB.97.126.C\_44-C6  
GGGAGAGGAGAGAACGTTCTCGGTACAAGGAACACAATAGTTAGTGCCGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 135 >PB.97.126.C\_44-D6  
GGGAGAGGAGAGAACGTTCTCGGAACCTCAGAGATCCTATGTGGACCAGAGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 136 >PB.97.126.C\_44-E6  
GGGAGAGGAGAGAACGTTCTCGCTGAGCAAGGAACGTAATAGTTAGCCTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 137 >PB.97.126.C\_44-F6  
GGGAGAGGAGAGAACGTTCTCGNANNNATAAATGATGGATCNCTTATTGTNNAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 138 >PB.97.126.C\_44-G6  
GGGAGAGGAGAGAACGTTCTCGGCTTGGA AAAATAGCTTTTGGGCATCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 139 >PB.97.126.C\_44-H6  
GGGAGAGGAGAGAACGTTCTCGGGTTCAAGGAACATGATAGCTAGAACCCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 140 >PB.97.126.C\_44-A7  
GGGAGAGGAGAGAACGTTCTCGGGTTCAAGGAACATGATAGTTAGAACCCGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 141 >PB.97.126.C\_44-B7  
GGGAGAGGAGAGAACGTTCTCGTGGGCAGGGAACACAATAGTTAGCCTACGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 142 >PB.97.126.C\_44-C7  
GGGAGAGGAGAGAACGTTCTCGCGTGAAAGGAACACAATAGTTATCGTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 143 >PB.97.126.C\_44-D7  
GGGAGAGGAGAGAACGTTCTCGCGAGGTTTATCCTAGACGACTAACCGCCTGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 144 >PB.97.126.C\_44-F7  
GGGAGAGGAGAGAACGTTCTCGTCTGCTAGGAACACAATAGTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 145 >PB.97.126.C\_44-G7  
GGGAGAGGAGAGAACGTTCTCGCACAAAGGAACTACGAGTTAGTGTGGGAGTGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 146 >PB.97.126.C\_44-H7  
GGGAGAGGAGAGAACGTTCTCGTGACACGAGGAACCTTAGAGTTAGTAGCACGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 147 >PB.97.126.C\_44-A8  
GGGAGAGGAGAGAACGTTCTCGGCGGCGAAGGAACACAATAGTTACGTCCCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 148 >PB.97.126.C\_44-B8  
GGGAGAGGAGAGAACGTTCTCGAGCCAA AAAAGCTGAAGTACTTTGGGCAGGGATCGTTACGACTAGCATCGATG

**[00206] TABLE 9 – Corresponding cDNAs of the Thrombin Aptamer Sequences – 2'-OMe AUGC (r/mGmH, each G has a 90% probability of having a 2'-OMe group incorporated therein)**

SEQ ID No. 149 >PB.97.126.D\_44-D8  
GGGAGAGGAGAGAACGTTCTCGGTACAAGGAACACAATAGTTAGTGCCGTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 150 >PB.97.126.D\_44-E8  
GGGAGAGGAGAGAACGTTCTCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 151 >PB.97.126.D\_44-G8  
GGGAGAGGAGAGAACGTTCTCGTGC GCAAGGAACACAATAGTTAGGGCGCGAGGATCGTTACGACTAGCATTGATG

SEQ ID No. 152 >PB.97.126.D\_44-H8  
GGGAGAGGAGAGAACGTTCTCGGAATGGAAGGAACACAATAGTTACCAGACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 153 >PB.97.126.D\_44-A9

GGGAGAGGAGAGAACGTTCTCGTCTGCAAGGAACACAATAGTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 154 >PB.97.126.D\_44-B9  
GGGAGAGGAGAGAACGTTCTCGAGACAAGACAGCTGGAGGACTAAGTCACGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 155 >PB.97.126.D\_44-C9  
GGGAGAGGAGAGAACGTTCTCGATGCCCGCAAAGGAACACGATAGTTATGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 156 >PB.97.126.D\_44-D9  
GGGAGAGGAGAGAACGTTCTCGTCTGNNAGGAACACAATATTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 157 >PB.97.126.D\_44-E9  
GGGAGAGGAGAGAACGTTCTCGAATGTGCGGAGCAGTATTGGTACACTTTTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 158 >PB.97.126.D\_44-F9  
GGGAGAGGAGAGAACGTTCTCGCCAAGGAACACAATAGTTAGGTGAGAATCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 159 >PB.97.126.D\_44-G9  
GGGAGAGGAGAGAACGTTCTCGCCAAGGAACACAATAGTTAGGTGAGAATCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 160 >PB.97.126.D\_44-H9  
GGGAGAGGAGAGAACGTTCTCGGAAGCAAGGAACCTTAGAGTTAGTTGACCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 161 >PB.97.126.D\_44-A10  
GGGAGAGGAGAGAACGTTCTCGTGGGCAAGGAACACAATAGTTAGCCTACGCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 162 >PB.97.126.D\_44-B10  
GGGAGAGGAGAGAACGTTCTCGTCGGGCATGGAACACAATAGTTAGACCGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 163 >PB.97.126.D\_44-C10  
GGGAGAGGAGAGAACGTTCTCGGTCGCAAGGAACATAATAGTTAGCGGAGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 164 >PB.97.126.D\_44-D10  
GGGAGAGGAGAGAACGTTCTCGTCTGCAAGGAACACAATAGTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 165 >PB.97.126.D\_44-E10  
GGGAGAGGAGAGAACGTTCTCGCCGACAAATCAGCTCGGATCGTGTGCTACGCTGGATCGTTACGACTAGCATCGATG

**[00207] TABLE 10 – Corresponding cDNAs of the Thrombin Aptamer Sequences –alternately “r/mGmH” and 2’-OMe AUC, 2’-F G (toggle).**

SEQ ID No. 166 >PB.97.126.E\_44-F10  
GGGAGAGGAGAGAACGTTCTCGAGACAAGATAGCTGAAGGACTAAGTCACGAGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 167 >PB.97.126.E\_44-G10  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTTCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 168 >PB.97.126.E\_44-H10  
GGGAGAGGAGAGAACGTTCTCGGAGNCAAGGAAACNAATATTTAGGCTCANTGGNNNCNTTNCANCTAGCNNCNCNTA

SEQ ID No. 169 >PB.97.126.E\_44-A11  
GGGAGAGGAGAGAACGTTCTCGTCTGCAAGGAACACAATAGTTAGCATTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 170 >PB.97.126.E\_44-B11  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 171 >PB.97.126.E\_44-C11  
GGGAGAGGAGAGAACGTTCTCGGATCGTTACGACTAGCATCGATG

SEQ ID No. 172 >PB.97.126.E\_44-D11  
GGGAGAGGAGAGAACGTTCTCGGTGATAGTACTCACATAGAAATGGCTACACTGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 173 >PB.97.126.E\_44-E11  
GGGAGAGGAGAGAACGTTCTCGCCTGGGCAAGGAACAGAAAAGTTAGCGCCAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 174 >PB.97.126.E\_44-F11  
GGGAGAGGAGAGAACGTTCTCGTAACGGACAAAAGGAACCGGGAAGTTATCTGGATCGTTACGACTAGCATCGATG

SEQ ID No. 175 >PB.97.126.E\_44-G11  
GGGAGAGGAGAGAACGTTCTCGCGCACAAAGATAGAGAAGACTAAGTCCGCGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 176 >PB.97.126.E\_44-H11  
GGGAGAGGAGAGAACGTTCTCGCGCACAAAGATAGAGAAGACTAAGTTCGCGGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 177 >PB.97.126.E\_44-A12  
GGGAGAGGAGAGAACGTTCTCGCGCCAATAAAGCTGGAGTACTTAGAGCGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 178 >PB.97.126.E\_44-B12  
GGGAGAGGAGAGAACGTTCTCGGGAACAAGGAACCTAGAGTTAGTTGACCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 179 >PB.97.126.E\_44-C12  
GGGAGAGGAGAGAACGTTCTCGCTAGCAAGATAGGTGGGACTAAGCTAGTGAGGATCGTTACGACTAGCATCGATG

SEQ ID No. 180 >PB.97.126.E\_44-D12  
GGGAGAGGAGAGAACGTTCTCGTCTGAAGGGGAGCTTTGTCTCGGGACAGAACGGATCGTTACGACTAGCATCGATG

SEQ ID No. 181 >PB.97.126.E\_44-E12  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTACGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 182 >PB.97.126.E\_44-G12  
GGGAGAGGAGAGAACGTTCTCGGAACAAGATAGCTGAAGGACTAAGTTTGCGGGATCGTTACGACTAGCATCGATG

SEQ ID No. 183 >PB.97.126.E\_44-H12  
GGGAGAGGAGANNTCCCNCCNCGGAAAAAANAAAAAGAAGAANTANGTTNGGGGGATCGTTACGACTAGCATCGATG

**[00208]** Table 11 – Stabilized Aptamer Sequences (each G residue has 90% probability of being substituted with a 2'-OMe group, "3T" refers to an inverted thymidine nucleotide attached to the phosphodiester backbone at the 5' position, the resulting oligo having two 5'-OH ends and is thus resistant to 3' nucleases).

SEQ ID No. 184 ARC224 -Stabilized VEGF Aptamer  
5' mCmGmAmUmAmUmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmAmUmUmCmG-3T

SEQ ID No. 185 ARC225 - Stabilized VEGF Aptamer  
5' mCmGmAmUmAmUGmCmAGmUmUmUGmAGmAmAGmUmCGmCGmCmAmUmUmCmG-3T

SEQ ID No. 186 ARC226 Single-hydroxy VEGF aptamer  
5' mGmAmUmCmAmUmGmCmAmUGmUmGmGmAmUmCmGmCmGmGmAmUmC-3T

SEQ ID No. 187 ARC245 VEGF Aptamer  
5' mAmUmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmAmU-3T

SEQ ID No, 188 ARC259 hVEGF Aptamer- C-G base pair swap of ARC245 (2nd base pair in) which has improved binding over ARC245.  
5' mAmCmGmCmAmGmUmUmUmGmAmGmAmAmGmUmCmGmCmGmCmGmU-3'

## Example 2    2'-OMe SELEX™

**[00209]** Libraries of transcription templates were used to generate pools of RNA oligonucleotides incorporating 2'-O-methyl NTPs under various transcription conditions. The transcription template (ARC256) and the transcription conditions are described below as rRmY (SEQ ID NO:456), rGmH (SEQ ID NO:462), r/mGmH (SEQ ID NO:463), and dRmY (SEQ ID NO:464). The unmodified RNA transcript is represented by SEQ ID NO:468.

ARC256: DNA transcription template

5'-CATCGATCGATCGATCGACAGCGNNNGTAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:453)

The ARC256 RNA transcription product is:

5'-GGGAGAGGAGAGAACGUUCUACNNNCGCUGUCGAUCGAUCGAUCGAUG-3' (SEQ ID NO:468)

**[00210]** The transcription conditions were varied as follows where 1X Tc buffer is 200 mM HEPES, 40 mM DTT, 2 mM Spermidine, 0.01% Triton X-100, pH 7.5.

**[00211]** When 2'-OMe C and U and 2'-OH A and G (rRmY) conditions were used, the transcription reaction conditions were 1X Tc buffer, 50-200 nM double stranded template (200 nm template was used for round 1, and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein, was used), 9.6 mM MgCl<sub>2</sub>, 2.9 mM MnCl<sub>2</sub>, 2 mM each base, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F/H784A T7 RNA polymerase. One unit of the Y639F/H784A mutant T7 RNA polymerase is defined as the amount of enzyme required to incorporate 1 nmole of 2'-OMe NTPs into transcripts under the r/mGmH conditions. One unit of inorganic pyrophosphatase is defined as the amount of enzyme that will liberate 1.0 mole of inorganic orthophosphate per minute at pH 7.2 and 25 °C.

**[00212]** When 2'-OMe A, C, and U and 2'-OH G (rGmH) conditions were used, the transcription reaction conditions were 1X Tc buffer, 50-200 nM double stranded DNA template (200 nm template was used for round 1, and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein was used), 9.6 mM

MgCl<sub>2</sub>, 2.9 mM MnCl<sub>2</sub>, 2 mM each base, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F single mutant T7 RNA polymerase. One unit of the Y639F mutant T7 RNA polymerase is defined as the amount of enzyme required to incorporate 1 nmole of 2'-OMe NTPs into transcripts under the r/mGmH conditions.

**[00213]** When all 2'-OMe nucleotides (r/mGmH) conditions were used, the reaction conditions were 1X Tc buffer, 50-200 nM double stranded template (200 nm template was used for round 1, and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein was used), 6.5 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 1 mM each base, 30 μM GTP, 1 mM GMP, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F/H784A T7 RNA polymerase.

**[00214]** When deoxy purines, A and G, and 2'-OMe pyrimidines (dRmY) conditions were used, the reaction conditions were 1X Tc buffer, 50-300 nM double stranded template (300 nm template was used for round 1, and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein was used), 9.6 mM MgCl<sub>2</sub>, 2.9 mM MnCl<sub>2</sub>, 2 mM each base, 30 μM GTP, 2 mM Spermine, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F single mutant RNA polymerase.

**[00215]** These pools were then used in SELEX™ to select for aptamers against the following targets: IgE, IL-23, PDGF-BB, thrombin and VEGF. A plot of dRmY Round 6, 7, 8, and unselected sequences binding to target IL-23 is shown in Figure 14, and a plot of dRmY Round 6, 7, and unselected sequences binding to target PDGF-BB is shown in Figure 14.

### Example 3     dRmY SELEX™ of Aptamers against IgE

**[00216]** While fully 2'-OMe substituted oligonucleotides are the most stable modified aptamers, substituting the purines with deoxy purine nucleotides also results in stable transcripts. When dRmY (deoxy purines, A and G, and 2'-OMe pyrimidines) transcription conditions are used, the products are very DNase-resistant and useful as stable therapeutics. This result is surprising since the composition of the dRmY transcripts is approximately 50% DNA, which is notoriously easily degraded by nucleases. Also, when dRmY transcription conditions are used, there is no requirement for a 2'-OH GTP spike. Studies have shown that approximately the same amount of dRmY transcripts having modified nucleotides are produced with 2'-OH GTP doping as without

2'-OH GTP doping. Accordingly, under dRmY transcription conditions, 2'-OH GTP doping is optional. Libraries of transcription templates were used to generate pools of oligonucleotides incorporating 2'-O-methyl pyrimidine NTPs (U and C) and deoxy purines (A and G) NTPs under various transcription conditions. The transcription template (ARC256) and the transcription conditions are described below as dRmY.

ARC256: DNA transcription template

5'-CATCGATCGATCGATCGACAGCGNNNGTAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:453)

The ARC256 dRmY RNA transcription product is:

5'-GGGAGAGGAGAGAACGUUCUACNNNCGCUGUCAUCGAUCGAUCGAUG-3' (SEQ ID NO:464)

**[00217]** When deoxy purines, A and G, and 2'-OMe pyrimidines (dRmY) conditions were used, the reaction conditions were 1X Tc buffer, 50-300 nM double stranded template (300 nm template was used for round 1, and for subsequent rounds approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein, was used), 9.6 mM MgCl<sub>2</sub>, 2.9 mM MnCl<sub>2</sub>, 2 mM each base, 30 μM GTP, 2 mM Spermine, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F single mutant RNA polymerase.

**[00218]** These pools were then used in SELEX™ to select for aptamers against IgE as a target. The sequences obtained after round 6 of SELEX™ as described above are listed in Table 12 below. A plot of Round 6 sequences bound with increasing target IgE concentration is shown in Figure 8.

**[00219]** Table 12 – Corresponding cDNAs of the Round 6 sequences of dRmY SELEX™ against IgE.

SEQ ID No.190 IgE A5

GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCGCTGTCGATCGATCGATCGATG

SEQ ID No.191 IgE A6

GGGAGAGGAGAGAACGTTCTACGATTAGCAGGGAGGGAGAGTGCGAAGAGGACGCTGTCGATCGATCGATCGATG

SEQ ID No.192 IgE A7

GGGAGAGGAGAGAACGTTCTACACTCTGGGGACCCGTGGGGGAGTGCGAGCAACGCTGTCGATCGATCGATCGATG



SEQ ID No.193 IgE A8  
GGGAGAGGAGAGAACGTTCTACAAGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.194 IgE B5  
GGGAGAGGAGAGAACGTTCTACGAGGTGAGGGTCTACAATGGAGGGATGGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.195 IgE B6  
GGGAGAGGAGAGAACGTTCTACCCGAGCATAGCCTGNGGACCCATGNGGGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.196 IgE B7  
GGGAGAGGAGAGAACGTTCTACTGGGGGCGTGTTCATTAGCAGCGTCGTGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.197 IgE B8  
GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.198 IgE C5  
GGGAGAGGAGAGAACGTTCTACGCAGCGCATCTGGGGACCCAAGAGGGGATTCGCTGTCGATCGATCGATCGATG

SEQ ID No.199 IgE C6  
GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.200 IgE C7  
GGGAGAGGAGAGAACGTTCTACGGGATGGGTAGTTGGATGGAAATGGGAACGCTGTCGATCGATCGATCGATG

SEQ ID No.201 IgE C8  
GGGAGAGGAGAGAACGTTCTACGAGGTGTAGGGATAGAGGGGTGTAGGTAACGCTGTCGATCGATCGATCGATG

SEQ ID No.202 IgE D5  
GGGAGAGGAGAGAACGTTCTACAGGAGTGGAGCTACAGAGAGGGTTAGGGGTGCTGTCGATCGATCGATCGATG

SEQ ID No.203 IgE D6  
GGGAGAGGAGAGAACGTTCTACGGATGTTGGGAGTGATAGAAGGAAGGGGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.204 IgE D7  
GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.205 IgE D8  
GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.206 IgE E5  
GGGAGAGGAGAGAACGTTCTACAGGCAGTTCTGGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.207 IgE E6  
GGGAGAGGAGAGAACGTTCTACTTGGGGTGAAGGAGTAAGGGAGGTGCTGATCGCTGTCGATCGATCGATCGATG

SEQ ID No.208 IgE E7  
GGGAGAGGAGAGAACGTTCTACGTATTAGGGGGGAAGGGGAGGAATAGATCACGCTGTCGATCGATCGATCGATG

SEQ ID No.209 IgE E8

GGGAGAGGAGAGAACGTTCTACAGGGAGAGAGTGTGAGTGAAGAGGAGAGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.210 IgE F5

GGGAGAGGAGAGAACGTTCTACATTGTGCTCCTGGGGCCAGTGGGGAGCCACGCTGTCGATCGATCGATCGATG

SEQ ID No.211 IgE F6

GGGAGAGGAGAGAACGTTCTACGAGCAGCCCTGGGGCCCGAGGGGGATGGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.212 IgE F7

GGGAGAGGAGAGAACGTTCTACAGGCAGTCTGCGGGACCCATGGGGGAAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.213 IgE F8

GGGAGAGGAGAGAACGTTCTACCAACGGCATCCTGGGGCCACAGGGGATGGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.214 IgE G5

GGGAGAGGAGAGAACGTTCTACGAGTGGATAGGAAGAAGGGGAGTAGTCACGCTGTCGATCGATCGATCGATG

SEQ ID No.215 IgE G6

GGGAGAGGAGAGAACGTTCTACCCGCAGCATAGCTGGGGACCCATGGGGGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.216 IgE G7

GGGAGAGGAGAGAACGTTCTACGGTCGCGTGTGGGGACGGATGGGTATTGGTCGCTGTCNATCGATCGATCNATG

SEQ ID No.217 IgE G8

GGGAGAGGAGAGAACGTTCTACCCGCAGCATAGCTGGGGACCCATGGGGGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.218 IgE H5

GGGAGAGGAGAGAACGTTCTACCCGCAGCATAGCTGGGGACCCATGGGGGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.219 IgE H6

GGGAGAGGAGAGAACGTTCTACGGGGTTACGTCGCACGATACATGCATTCATCGCTGTCGATCGATCGATCGATG

SEQ ID No.220 IgE H7

GGGAGAGGAGAGAACGTTCTACTAGCGAGGAGGGGTTTTCTATTTTTCGATCGCTGTCGATCGATCGATCGATG

#### Example 4 dRmY SELEX™ of Aptamers against Thrombin

[00220] While fully 2'-OMe substituted oligonucleotides are the most stable modified aptamers, substituting the purines with deoxy purine nucleotides also results in stable transcripts. When dRmY (deoxy purines, A and G, and 2'-OMe pyrimidines) transcription conditions are used, the products are very DNase-resistant and useful as stable therapeutics. This result is surprising since the composition of the dRmY transcripts is approximately 50% DNA, which is notoriously easily degraded by nucleases. Also, when dRmY transcription conditions are used, there is no requirement for a 2'-OH GTP spike. Libraries of transcription templates were used to generate pools of oligonucleotides incorporating 2'-O-methyl pyrimidine NTPs (U and C) and deoxy



SEQ ID No.225 Thrombin B1  
GGGAGAGGAGAGAACGTTCTACAGGTAGCGTGAGGGGGTGTTAATAGAGGGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.226 Thrombin B2  
GGGAGAGGAGAGAACGTTCTACGATAGGATGGGTGGGACAGGAGAGGGAGTGCCTGTCGATCGATCGATCGATG

SEQ ID No.227 Thrombin B3  
GGGAGAGGAGAGAACGTTCTACAGTGAGGGCAGTGTGAGATTGAGAGGAGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.228 Thrombin B4  
GGGAGAGGAGAGAACGTTCTACCTTGCCTAACAGGAGGTGGAGTATTGGACCCGCTGTCGATCGATCGATCGATG

SEQ ID No.229 Thrombin C1  
GGGAGAGGAGAGAACGTTCTACCTTGCCTAACAGGAGGTGGAGTATTGGACCCGCTGTCGATCGATCGATCGATG

SEQ ID No.230 Thrombin C2  
GGGAGAGGAGAGAACGTTCTACGTCGTGAGTAATGGCTCGTAGATGAGGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.231 Thrombin C3  
GGGAGAGGAGAGAACGTTCTACGGGATTAAGAGGGGAGAGGAGCAGTTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.232 Thrombin C4  
GGGAGAGGAGAGAACGTTCTACTCCGGTTGGGGTATCAGGTCTACGGACTGACGCTGTCGATCGATCGATCGATG

SEQ ID No.233 Thrombin D1  
GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.234 Thrombin D2  
GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.235 Thrombin D3  
GGGAGAGGAGAGAACGTTCTACATGACAAGAGGGGGTTGTGTGGGATGGCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.236 Thrombin D4  
GGGAGAGGAGAGAACGTTCTACACAGGGAGGGGAGCGGAGAGGAGAGAGGGTACGCTGTCGATCGATCGATCGATG

SEQ ID No.237 Thrombin E1  
GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.238 Thrombin E2  
GGGAGAGGAGAGAACGTTCTACGTCGTGAGTAATGGCTCGTAGATGAGGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.239 Thrombin E4  
GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.240 Thrombin F1  
GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.241 Thrombin F2  
GGGAGAGGAGAGAACGTTCTACCTTGCCTAACAGGAGGTGGAGTATTGGACCCGCTGTCGATCGATCGATCGATG

SEQ ID No.242 Thrombin F3

GGGAGAGGAGAGAACGTTCTACGGCTATGCGTCGTGAGTCAATGGCCCGCATCGCTGTCGATCGATCGATCGATG

SEQ ID No.243 Thrombin F4

GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATACTGGCTCCCGTATTTCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.244 Thrombin G1

GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTTCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.245 Thrombin G2

GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTTCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.246 Thrombin G3

GGGAGAGGAGAGAACGTTCTACCTTGTCTAACAGGAGGTGGAGTATTGGACCCGCTGTCGATCGATCGATCGATG

SEQ ID No.247 Thrombin G4

GGGAGAGGAGAGAACGTTCTACGACTTTGAGGGTGGTGAGAGTGAAGAGAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.248 Thrombin H1

GGGAGAGGAGAGAACGTTCTACGGTAGGGTATGACCAGGGAGGTATTGGAGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.249 Thrombin H2

GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTTCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.250 Thrombin H3

GGGAGAGGAGAGAACGTTCTACGGGTCGTGAGATAATGGCTCCCGTATTTCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.251 Thrombin H4

GGGAGAGGAGAGAACGTTCTACGTTATGCATGTGGAGAGTGAGAGAGGGCGCTGTCGATCGATCGATCGATG

### Example 5 dRmY SELEX™ of Aptamers against VEGF

[00224] While fully 2'-OMe substituted oligonucleotides are the most stable modified aptamers, substituting the purines with deoxy purine nucleotides also results in stable transcripts. When dRmY (deoxy purines, A and G, and 2'-OMe pyrimidines) transcription conditions are used, the products are very DNase-resistant and useful as stable therapeutics. This result is surprising since the composition of the dRmY transcripts is approximately 50% DNA RNA, which is notoriously easily degraded by nucleases. Also, when dRmY transcription conditions are used, there is no requirement for a 2'-OH GTP spike. Libraries of transcription templates were used to generate pools of oligonucleotides incorporating 2'-O-methyl pyrimidine NTPs (U and C) and deoxy purines (A and G) NTPs under various transcription conditions. The transcription template (ARC256) and the transcription conditions are described below as dRmY.

### ARC256: DNA transcription template

5'-CATCGATCGATCGATCGACAGCGNNNGTAGAACGTTCTCTCCTCTCCCTATAGTGAGTCGTATTA-3' (SEQ ID NO:453)

ARC256 dRmY transcription product is:

5'-GGGAGAGGAGAGAACGUUCUACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN  
NNNNCGCUGUCGAUCGAUCGAUCGAUG-3' (SEQ ID NO:464)

**[00225]** When deoxy purines, A and G, and 2'-OMe pyrimidines (dRmY) conditions were used, the reaction conditions were 1X Tc buffer, 50-300 nM double stranded template (300 nm template was used for round 1, and for subsequent rounds a 1/10 dilution of an optimized PCR reaction, using conditions described herein, was used), 9.6 mM MgCl<sub>2</sub>, 2.9 mM MnCl<sub>2</sub>, 2 mM each base, 30 μM GTP, 2 mM Spermine, 10% PEG-8000, 0.25 units inorganic pyrophosphatase, and 1.5 units Y639F single mutant RNA polymerase.

**[00226]** These pools were then used in SELEX™ to select for aptamers against VEGF as a target. The sequences obtained after round 6 of SELEX™ as described above are listed in an alignment show in Table 14 below. A plot of Round 6 sequences bound to target VEGF is shown in Figure 10.

**[00227]** Table 14 – Corresponding cDNAs of the Round 6 sequences of dRmY SELEX™ against VEGF.

SEQ ID No.252 VEGF A9

**GGGAGAGGAGAGAACGTTCTACCATGTCTGCGGGAGGTGAGTAGTGATCCTGCGCTGTCGATCGATCGATCGATCG**

SEQ ID No.253 VEGF A10

GGGAGAGGAGAGAACGTTCTACAGAGTGGGAGGGATGTGTGACACAGGTAGGCGCTGTTCGATCGATCGATCGATCGATCG

SEQ ID No.254 VEGF A11

GGGAGAGGAGAGAACGTTCTACGCTCCATGACAGTGAGGTGAGTAGTGATCGCTGTCGATCGATCGATCGATG

SEQ ID No.255 VEGF A12

GGGAGAGGAGAGAACGTTCT CGATGCTGACAGGGTGTGTTTCAGTAATGGCTCGCTGTCGATCGATCGATCGATCG

SEO ID No.256 VEGF B9

GGGAGAGGAGAGAACGTTCTACCAGCAAACAGGGTCAGGTGAGTAGTGATGACGCTGTCGATCGATCGATCGATG

SEQ ID No.257 VEGF B10

GGGAGAGGAGAGAACGTTCTACGACAAGCCGGGGGTGTTTCAGTAGTGGCAACCGCTGTCGATCGATCGATCGATG

SEQ ID No.258 VEGF B11

GGGAGAGGAGAGAACGTTCTACATATGGCGCTGGAGGTGAGTAATGATCGTGCGCTGTCGATCGATCGATCGATG

SEQ ID No.259 VEGF B12

GGGAGAGGAGAGAACGTTCTACGGGGCGATAGCGTTCAGTAGTGGCGCCGGTTCGCTGTCGATCGATCGATCGATG

SEQ ID No.260 VEGF C9

GGGAGAGGAGAGAACGTTCTACATAGCGGACTGGGTGCATGGAGCGGCGCACGCTGTCGATCGATCGATCGATG

SEQ ID No.261 VEGF C10

GGGAGAGGAGAGAACGTTCTACGGGTCAACAGGGGCGTTCAGTAGTGGCGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.262 VEGF C11

GGGAGAGGAGAGAACGTTCTACGCATGCGAGCTGAGGTGAGTAGTGATCAGTCGCTGTCGATCGATCGATCGATG

SEQ ID No.263 VEGF C12

GGGAGAGGAGAGAACGTTCTACATGCGACAGGGGAGTGTTTCAGTAGTGGCACGCTGTCGATCGATCGATCGATG

SEQ ID No.264 VEGF D9

GGGAGAGGAGAGAACGTTCTACCCCATCGTATGGAGTGCGGAACGGGCATACGCTGTCGATCGATCGATCGATG

SEQ ID No.265 VEGF D10

GGGAGAGGAGAGAACGTTCTACAGTGAGGCGGGAGCGTTTCAGTAATGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.266 VEGF D12

GGGAGAGGAGAGAACGTTCTACACAGCGTCGGGTGTTTCAGTAATGGCGCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.267 VEGF E9

GGGAGAGGAGAGAACGTTCTACGGTGTTTCAGTAGTGGCACAGGAGGAAGGGATGCTGTCGATCGATCGATCGATG

SEQ ID No.268 VEGF E10

GGGAGAGGAGAGAACGTTCTACAGTTCAGGCGTTAGGCATGGGTGTCGCTTTCGCTGTCGATCGATCGATCGATG

SEQ ID No.269 VEGF E11

GGGAGAGGAGAGAACGTTCTACATGCGACATGCGAGTGTTTCAGTAGCGGCAGCGCTGTCGATCGATCGATCGATG

SEQ ID No.270 VEGF E12

GGGAGAGGAGAGAACGTTCTACCTATGGCGTTACAGCGAGGTGAGTAGTGATCGCTGTCGATCGATCGATCGATG

SEQ ID No.271 VEGF F9

GGGAGAGGAGAGAACGTTCTACAGCCGATCCAGCCAGGCGTTCAGTAGTGGCGCTGTCGATCGATCGATCGATG

SEQ ID No.272 VEGF F10

GGGAGAGGAGAGAACGTTCTACGGCACAGGCACGGCGAGGTGAGTAATGATCGCTGTCGATCGATCGATCGATG

SEQ ID No.273 VEGF G9

GGGAGAGGAGAGAACGTTCTACTGTGGACAGCGGGAGTGCGGAACGGGGTTCGCTGTCGATCGATCGATCGATG

SEQ ID No.274 VEGF G10

GGGAGAGGAGAGAACGTTCTACTGATGCTGCGAGTGCATGGGGCAGGCGCTTCGCTGTCGATCGATCGATCGATG

SEQ ID No.275 VEGF G11

GGGAGAGGAGAGAACGTTCTACGCTACAATGGGAATGACAGTGCATGGGTAGCCGCTGTCGATCGATCGATCGATG

SEQ ID No.276 VEGF G12

GGGAGAGGAGAGAACGTTCTACATGGACAGCGAAGCATGGGGGAGGCGCACGCTGTCGATCGATCGATCGATG

SEQ ID No.277 VEGF H9

GGGAGAGGAGAGAACGTTCTACTGGGAGCGACAGTGCATGGGGTAGGCGCCGCTGTCGATCGATCGATCGATG

SEQ ID No.278 VEGF H11

GGGAGAGGAGAGAACGTTCTACCGCGAGCAGGTGTTCACTAGTGGCTTTGCGCTGTCGATCGATCGATCGATG

SEQ ID No.279 VEGF H12

GGGAGAGGAGAGAACGTTCTACGATCAGTGAGGGAGTGCAGTAGTGGCTCGTCGCTGTCGATCGATCGATCGATG

#### Example 6 Plasma stability of 2'-OMe NTPs (mN) and dRmY oligonucleotides

[00228] An oligonucleotide of two sequences linked by a polyethylene glycol polymer (PEG) was synthesized in two versions: (1) with all 2'-OMe NTPs (mN): 5'-GGAGCAGCACC-3' (SEQ ID NO:457) -[PEG]- GGUGCCAAGUCGUUGCUC-3' (SEQ ID NO:458) and (2) with 2'-OH purine NTPs and 2'-OMe pyrimidines (dRmY) GGAGCAGCACC-3' (SEQ ID NO:465) -[PEG]- GGUGCCAAGUCGUUGCUC-3' (SEQ ID NO:466). These oligonucleotides were evaluated for full length stability. Figure 11A shows a degradation plot of the all 2'-OMe oligonucleotide with 3'idT and Figure 11B shows a degradation plot of the dRmY oligonucleotide. The oligonucleotides were incubated at 50 nM in 95% rat plasma at 37 °C and show a plasma half-life of much greater than 48 hours for each, and that they have very similar plasma stability profiles.

#### Example 7 rRmY and rGmH 2'-OMe SELEX™ against Human IL-23

[00229] Selections were performed to identify aptamers containing 2'-OMe C, U and 2'-OH G, A (rRmY), and 2'-O-Methyl A, C, and U and 2'-OH G (rGmH). All selections were direct selections against human IL-23 protein target which had been immobilized on a hydrophobic plate. Selections yielded pools significantly enriched for h-IL-23 binding versus naïve, unselected pool. Individual clone sequences for h-IL-23 are reported herein, but h-IL-23 binding data for the individual clones are not shown.



**[00230] Pool Preparation.** A DNA template with the sequence 5'–  
GGGAGAGGAGAGAACGTTCTACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCG  
CTGTCGATCGATCGATCGATG–3' (SEQ ID NO:459) was synthesized using an ABI  
EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The templates were  
amplified with the primers PB.118.95.G: 5'-GGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID  
NO:460) and STC.104.102.A (5'-CATCGATCGATCGATCGACAGC-3' (SEQ ID NO:461) and  
then used as a template (200 nm template was used for round 1, and for subsequent rounds  
approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described  
herein, was used) for in vitro transcription with Y639F single mutant T7 RNA polymerase.  
Transcriptions were done using 200 mM HEPES, 40 mM DTT, 2 mM spermidine, 0.01%  
TritonX-100, 10% PEG-8000, 5 mM MgCl<sub>2</sub>, 1.5 mM MnCl<sub>2</sub>, 500 μM NTPs, 500 μM GMP, 0.01  
units/μl inorganic pyrophosphatase, and Y639F single mutant T7 polymerase. Two different  
compositions were transcribed rRmY and rGmH.

**[00231] Selection.** Each round of selection was initiated by immobilizing 20 pmoles of h-IL-23  
to the surface Nunc Maxisorp hydrophobic plates for 2 hours at room temperature in 100 μL of  
1X Dulbecco's PBS. The supernatant was then removed and the wells were washed 4 times with  
120 μL wash buffer (1X DPBS, 0.2% BSA, and 0.05% Tween-20). Pool RNA was heated to  
90 °C for 3 minutes and cooled to room temperature for 10 minutes to refold. In round 1, a  
positive selection step was conducted. Briefly, 1 X 10<sup>14</sup> molecules (0.2 nmoles) of pool RNA  
were incubated in 100 μL binding buffer (1X DPBS and 0.05% Tween-20) in the wells with  
immobilized protein target for 1 hour. The supernatant was then removed and the wells were  
washed 4X with 120 μL wash buffer. In subsequent rounds a negative selection step was  
included. The pool RNA was also incubated for 30 minutes at room temperature in empty wells  
to remove any plastic binding sequences from the pool before the positive selection step. The  
number of washes was increased after round 4 to increase stringency. In all cases, the pool RNA  
bound to immobilized h-IL-23 was reverse transcribed directly in the selection plate after by the  
addition of RT mix (3' primer, STC.104.102.A, and Thermoscript RT, Invitrogen) followed by  
incubated at 65 °C for 1 hour. The resulting cDNA was used as a template for PCR (Taq  
polymerase, New England Biolabs) "Hot start" PCR conditions coupled with a 60 °C annealing  
temperature were used to minimize primer-dimer formation. Amplified pool template DNA was

desalted with a Centriscap column according to the manufacturer's recommended conditions and used to program transcription of the pool RNA for the next round of selection. The transcribed pool was gel purified on a 10 % polyacrylamide gel every round. Table 15 shows the RNA pool concentrations used per round of selection.

[00232] Table 15. RNA pool concentrations per round of selection.

pmoles Pool used	rRmY 2OMe				rGmH 3OMe			
Round	IL23	hIgE	mIgE	PDGF- BB	IL23	hIgE	mIgE	PDGF- BB
1	200	200	200	200	200	200	200	200
2	110	140	130	135	40	50	40	60
3	65	115	60	160	100	190	90	160
4	50	40	40	39	170	120	40	240
5	80	130	130	110	100	60	40	70
4	100	80	90	39	110	140	90	90
7	50	90	130	170	70	80	130	90
8	120		190	150	60	90	110	130
9	120		210	170	80	80	100	100
10	130		210	180				
11	110			210				

[00233] The selection progress was monitored using a sandwich filter binding assay. The 5'-<sup>32</sup>P-labeled pool RNA was refolded at 90 °C for 3 minutes and cooled to room temperature for 10 minutes. Next, pool RNA (trace concentration) was incubated with h-IL-23 DPBS plus 0.1 mg/ml tRNA for 30 minutes at room temperature and then applied to a nitrocellulose and nylon filter sandwich in a dot blot apparatus (Schleicher and Schuell). The percentage of pool RNA bound to the nitrocellulose was calculated and monitored approximately every 3 rounds with a single point screen (+/- 250 nM h-IL-23). Pool K<sub>D</sub> measurements were measured using a titration of protein and the dot blot apparatus as described above.

[00234] **Selection.** The rRmY h-IL-23 selection was enriched for h-IL-23 binding vs. the naïve pool after 4 rounds of selection. The selection stringency was increased and the selection was continued for 8 more rounds. At round 9 the pool K<sub>D</sub> was approximately 500 nM or higher. The rGmH selection was enriched over the naïve pool binding at round 10. The pool K<sub>D</sub> is also

approximately 500 nM or higher. The pools were cloned using TOPO TA cloning kit (Invitrogen) and individual sequences were generated. Figure 12 shows pool binding data to h-IL-23 for the rGmH round 10 and rRmY round 12 pools. Dissociation constants were estimated fitting data to the equation: fraction RNA bound = amplitude\* $K_D/(K_D + [h\text{-IL-23}])$ . Table 16 shows the individual clone sequences for round 12 of the rRmY selection. There is one group of 6 duplicate sequences and 4 pairs of 2 duplicate sequences out of 48 clones. All 48 clones will be labeled and tested for binding to 200 mM h-IL-23. Table 17 shows the individual clone sequences for round 10 of the rGmH selection. Binding data is shown in Figure 14.

[00235] Table 16. Corresponding cDNAs of the Individual Clone Sequences for Round 12 of the rRmY Selection.

```
SEQ ID No.280 ARX34P2.G01
GGGAGAGGAGAGAACGTTCTACAAATGAGAGCAGGCCGAAAAGGAGTCGCTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.281 ARX34P2.A06
GGGAGAGGAGAGAACGTTCTACAAAGGATCAATCTTTCGGCGTATGTGTGAGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.282 ARX34P2.E02
GGGAGAGGAGAGAACGTTCTACGGTAAAGCAGGCTGACTGAAAGGTTGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.283 ARX34P2.H05
GGGAGAGGAGAGAACGTTCTACAGGTTAAAGCAGGCTCAGGAATGGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.284 ARX34P2.G04
GGGAGAGGAGAGAACGTTCTACAAACAAAGCAGGCTCATAGTAATATGGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.285 ARX34P2.G03
GGGAGAGGAGAGAACGTTCTACAAAGAGAGCAGGCCGAAAAGGAGTCGCTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.286 ARX34P2.H06
GGGAGAGGAGAGAACGTTCTACAAAGGCAGGCTCAGGGGATCACTGGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.287 ARX34P2.B01
GGGAGAGGAGAGAACGTTCTACAAAAGCAGGCCGTATGGATATAAGGGAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.288 ARX34P2.B03
GGGAGAGGAGAGAACGTTCTACAAAGTGCAAGGCTGCAGACATATGCGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.289 ARX34P2.D05
GGGAGAGGAGAGAACGTTCTACAAAGGAGAGCAGGCCGAAAAGGAGTCGCTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.290 ARX34P2.C05
GGGAGAGGAGAGAACGTTCTACAAGATATAATTAAGGATAAGTGCAAAGGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.291 ARX34P2.C04
GGGAGAGGAGAGAACGTTCTACAGACAACAGCNAGAGGGAATCNCANACAAAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.292 ARX34P2.E06
GGGAGAGGAGAGAACGTTCTACAGATTCTAAGCGCAGGAATAAGTCACCAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.293 ARX34P2.A01
GGGAGAGGAGAGAACGTTCTACGAAAATGAGCATGGAAGTGGGAGTACGTGCCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.294 ARX34P2.C06
GGGAGAGGAGAGAACGTTCTACGAAAAGAGGCCCGGAAGTGAGAGTAAGTGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.295 ARX34P2.B04
GGGAGAGGAGAGAACGTTCTACGAAGTGAGTTTCCGAAGTGAGAGTACGAAACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.296 ARX34P2.E04
GGGAGAGGAGAGAACGTTCTACGAATGAGAGCAGGCCGAAAAGGAGTCGCTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.297 ARX34P2.H04
GGGAGAGGAGAGAACGTTCTACGAGAGGCAAGAGAGAGTCGCATAAAAAAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.298 ARX34P2.B06
GGGAGAGGAGAGAACGTTCTACGCAGGCTGTCGTAGACAAACGATGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.299 ARX34P2.F05
GGGAGAGGAGAGAACGTTCTACGGAAAAGATATGAAAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.300 ARX34P2.H02
GGGAGAGGAGAGAACGTTCTACGGAAAGGNAACAANAGCACTGTTTGTGCAAGGCGCTGTCGATCNATCNATCNATGAAGGGCG
SEQ ID No.301 ARX34P2.C03
GGGAGAGGAGAGAACGTTCTACGGAGCATANGGNTGAAACTGAGANAGTAACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.302 ARX34P2.D01
GGGAGAGGAGAGAACGTTCTACGAAAAGGATATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
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SEQ ID No.303 ARX34P2.A03  
 GGGAGAGGAGAGAACGTTCTACATACATAGGCGCCGCAATGGGAAAGAAAGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.304 ARX34P2.B02  
 GGGAGAGGAGAGAACGTTCTACTCATGAAGCCATGTTTGTAACTCTGTTTGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.305 ARX34P2.C01  
 GGGAGAGGAGAGAACGTTCTACTAATGCAGGCTCAGTTACTACTGGAAGTCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.306 ARX34P2.D06  
 GGGAGAGGAGAGAACGTTCTACTTTTCATAGCGGGATTATGGAGGAGTATTCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.307 ARX34P2.G05  
 AGGAGAGGAGAGAACGTTCTACTAGAAGCAGGCTCGAATAACAATTGGAAGTCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.308 ARX34P2.F06  
 GGGAGAGGAGAGAACGTTCTACTTAGCGATGTCGGAAGAGAGAGTACGAGGACGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.309 ARX34P2.F02  
 GGGAGAGGAGAGAACGTTCTACTTGCAGAACCGTGAAGAGGAGTACTGGTCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.310 ARX34P2.B05  
 GGGAGAGGAGAGAACGTTCTACTTTTGGTGAAGGTGAAGAGTGGCACTACACGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.311 ARX34P2.A05  
 GGGAGAGGAGAGAACGTTCTACCATCAGTTGTGGCGATTATGTGGGAGTATGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.312 ARX34P2.E03  
 GGGAGAGGAGAGAACGTTCTACANAANAACATGCGATTAAAGATCATGAACAGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.313 ARX34P2.F04  
 GGGAGAGGAGAGAACGTTCTACATAAGCAGGCTCCGATAGTATTCGGAAGTCGCTGTGATCGATCGATCGATCGATGAAGGGCG

[00236] Table 17. Corresponding cDNAs of the Individual Clone Sequences for Round 10 of the rGmH Selection.

SEQ ID No.314 ARX34P2.E10  
 GGGAGAGGAGAGAACGTTCTACTTTTCGGAATGCGATGGGGGTGATTCTGGTGTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.315 ARX34P2.H09  
 GGGAGAGGAGAGAACGTTCTACCTGTTGAGGCTAAGTGGATGATTGAGGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.316 ARX34P2.A07  
 GGGAGAGGAGAGAACGTTCTACTGCGTTCGATGCGATTGGAGATGTCGTTGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.317 ARX34P2.A12  
 GGGAGAGGAGAGAACGTTCTACCTGATGTCAGGTTGTTTGGAGATTATCTGACNCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.318 ARX34P2.A08  
 GGGAGAGGAGAGAACGTTCTACCTCGCGACGAGCGAATTTCCGGATGCGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.319 ARX34P2.D12  
 GGGAGAGGAGAGAACGTTCTACCATGAATGATTGCGATCGTTGTTGCTGTGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.320 ARX34P2.E11  
 GGGAGAGGAGAGAACGTTCTACTCCGACCGCTGGGTGATTCTACNACGACGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.321 ARX34P2.E12  
 GGGAGAGGAGAGAACGTTCTACTACTTTTGGGGATTCACTCCGCGCTGATGTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.322 ARX34P2.D08  
 GGGAGAGGAGAGANAACGTTCTANTAGTGCTTGCAGATAGTGATGATTATCTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.323 ARX34P2.F07  
 GGGAGAGGAGAGAACGTTCTACTAGTGCTCTTCTCCACGTTGGTTGTAATTGTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.324 ARX34P2.B11  
 GGGAGAGGAGAGAACGTTCTACTATTGTGGCGCTTGTGGACTAACTGACTACGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.325 ARX34P2.F12  
 GGGAGAGGAGAGAACGTTCTACTTCGATTGTGATCTTGTGGCGGCTGTGAGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.326 ARX34P2.A09  
 GGGAGAGGAGAGAACGTTCTACTTGGCGATGTCGGAAGAGAGAGTACGAGGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.467 ARX34P2.B07  
 GGGAGAGGAGAGAACGTTCTACTGTGACGACGGGCTTGAGAGGCTCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.327 ARX34P2.D07  
 GGGAGAGGAGAGAACGTTCTACTTGAANCTGCGTGAATTGANAGTAACGAAGCGCTGTCAATCGATCNATCAATNAAGGGCG  
 SEQ ID No.328 ARX34P2.H10  
 GGGAGAGGAGAGAACGTTCTACTCGAGAGGACATGTGGATCCGGTTCGCGTGCCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.329 ARX34P2.H07  
 GGGAGAGGAGAGAACGTTCTACTGTGATGCGGTTTGCCTGACCGGATTCGCTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.330 ARX34P2.F11  
 GGGAGAGGAGAGAACGTTCTACTGTGATTGGGCGCATGTCGAGGCGACACGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.331 ARX34P2.G07  
 GGGAGAGGAGAGAACGTTCTACTGATTAAGATGCGCTGGTAGACGGTGGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.332 ARX34P2.A10  
 GGGAGAGGAGAGAACGTTCTACTGGTTAATTTGCATGCGCGANTAACNTGNTGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.333 ARX34P2.G10  
 GGGAGAGGAGAGAACGTTCTACTGGGAAGCGGTAACCTGGATTGACCGATCCGCTGTGATCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.334 ARX34P2.H11  
 GGGAGAGGAGAGAACGTTCTACTGTACGAGATGATGGGTTTGGCTGTTGGCGCTGTGATCGATCGATCGATCGATGAAGGGCG

SEQ ID No.335 ARX34P2.C07  
 GGGAGAGGAGAGAACGTTCTACTTGTGGACTGAGATACGATTCCGGAGCTGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.336 ARX34P2.E08  
 GGGAGAGGAGAGAACGTTCTACTTGTGAGTTTCTTGGGCCCTTGAGCGTGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.337 ARX34P2.A11  
 GGGAGAGGAGAGAACGTTCTACAGGTGATGTGAGCCGATTGTGAAGTTTTGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.338 ARX34P2.B08  
 GGGAGAGGAGAGAACGTTCTACAGCGGATGTTTGGGGGTGTGTGTTGGTTGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.339 ARX34P2.B09  
 GGGAGAGGAGAGAACGTTCTACATCGGTGGTGGTCTTCGATGGGTGGAAGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.340 ARX34P2.B12  
 GGGAGAGGAGAGAACGTTCTACATTGGAGGGGCGCATGTGGTCTGTTTGTGATGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.341 ARX34P2.F10  
 GGGAGAGGAGAGAACGTTCTACGTGTTTCGCGGATTTGAAGAGGAGTAAATCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.342 ARX34P2.B10  
 GGGAGAGGAGAGAACGTTCTACGTGTGCGTGTTCGGGAAGGGAGAGTGCCGAGGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.343 ARX34P2.G08  
 GGGAGAGGAGAGAACGTTCTACGTGTGCGTGTTCGGGATGCTTGGCTGTTTGTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.344 ARX34P2.C08  
 GGGAGAGGAGAGAACGTTCTACGTTTGTGTGGCTTGGATCTGAAGACTAAGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.345 ARX34P2.F09  
 GGGAGAGGAGAGAACGTTCTACGTTTCTGGGCTTGTGTGTGAGGATTGACGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.346 ARX34P2.C10  
 GGGAGAGGAGAGAACGTTCTACGATGATGAAGGCGAAAAGACGAGGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.347 ARX34P2.C11  
 GGGAGAGGAGAGAACGTTCTACGAGTCTGATGCGTGTCTGGGATGGAATTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.348 ARX34P2.D09  
 GGGAGAGGAGAGAACGTTCTACGCTTTATAGCGATCGATGATGATATAGCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.349 ARX34P2.D10  
 GGGAGAGGAGAGAACGTTCTACGCTTCAAATGGGATAGAATTGGCTGCGGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.350 ARX34P2.D11  
 GGGAGAGGAGAGAACGTTCTACGAAATTGTGCGTCAGTGTGAGGCGGTTTGTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.351 ARX34P2.E07  
 GGGAGAGGAGAGAACGTTCTACGTCGAAATGAGGGTCTGGAGTTCCGACGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.352 ARX34P2.E09  
 GGGAGAGGAGAGAACGTTCTACGAAATTGGTAATCTGGGTGACTTAGGATGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.353 ARX34P2.G12  
 GGGAGAGGAGAGAACGTTCTACGATTTTGTGCGCAAGTAAGAGTACGCGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.354 ARX34P2.H08  
 AGGAGAGGAGAGAACGTTCTACGGAGTGTGCGCGGATGAAACAGAAGTTGTCGCTGTCNATCGATCNATCAATGAAGGGCG

#### Example 8 rRmY 2'-OMe SELEX™ against Human IgE

[00237] Selections were performed to identify aptamers containing 2'-OMe C, U and 2'-OH G, A (rRmY). All selections were direct selections against human IgE protein target which had been immobilized on a hydrophobic plate. Selections yielded pools significantly enriched for h-IgE binding versus naïve, unselected pool. Individual clone sequences for h-IgE are reported herein, but h-IgE binding data for the individual clones are not shown.

[00238] **Pool Preparation.** A DNA template with the sequence 5'–

GGGAGAGGAGAGAACGTTCTACNNCG  
 CTGTCGATCGATCGATCGATG–3' (SEQ ID NO:459) was synthesized using an ABI  
 EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The templates were  
 amplified with the primers PB.118.95.G 5'-GGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID  
 NO:460) and STC.104.102.A 5'-CATCGATCGATCGATCGACAGC-3' (SEQ ID NO:461) and  
 then used as a template (200 nm template was used for round 1, and for subsequent rounds

approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described herein, was used) for *in vitro* transcription with Y639F single mutant T7 RNA polymerase. Transcriptions were done using 200 mM HEPES, 40 mM DTT, 2 mM spermidine, 0.01% TritonX-100, 10% PEG-8000, 5 mM MgCl<sub>2</sub>, 1.5 mM MnCl<sub>2</sub>, 500 μM NTPs, 500 μM GMP, 0.01 units/μL inorganic pyrophosphatase, and Y639F single mutant T7 polymerase.

**Selection.** Each round of selection was initiated by immobilizing 20 pmoles of h-IgE to the surface Nunc Maxisorp hydrophobic plates for 2 hours at room temperature in 100 μL of 1X Dulbecco's PBS. The supernatant was then removed and the wells were washed 4 times with 120 μL wash buffer (1X DPBS, 0.2% BSA, and 0.05% Tween-20). Pool RNA was heated to 90 °C for 3 minutes and cooled to room temperature for 10 minutes to refold. In round 1, a positive selection step was conducted. Briefly,  $1 \times 10^{14}$  molecules (0.2 nmoles) of pool RNA were incubated in 100 μL binding buffer (1X DPBS and 0.05% Tween-20) in the wells with immobilized protein target for 1 hour. The supernatant was then removed and the wells were washed 4X with 120 μL wash buffer. In subsequent rounds a negative selection step was included. The pool RNA was also incubated for 30 minutes at room temperature in empty wells to remove any plastic binding sequences from the pool before the positive selection step. The number of washes was increased after round 4 to increase stringency. In all cases, the pool RNA bound to immobilized h-IgE was reverse transcribed directly in the selection plate after by the addition of RT mix (3' primer, STC.104.102.A, and Thermoscript RT, Invitrogen) followed by incubated at 65 °C for 1 hour. The resulting cDNA was used as a template for PCR (Taq polymerase, New England Biolabs) "Hot start" PCR conditions coupled with a 60 °C annealing temperature were used to minimize primer-dimer formation. Amplified pool template DNA was desalted with a Centriscp column according to the manufacturer's recommended conditions and used to program transcription of the pool RNA for the next round of selection. The transcribed pool was gel purified on a 10 % polyacrylamide gel every round.

[00239] rRmY pool selection against h-IgE was enriched after 4 rounds over the naïve pool. The selection stringency was increased and the selection was continued for 2 more rounds. At round 6 the pool K<sub>D</sub> is approximately 500 nM or higher. The pools were cloned using TOPO TA cloning kit (Invitrogen) and submitted for sequencing. The pool contained one dominant clone (AMX(123).A1)- which made up 71% of the clones sequenced. Three additional clones were

tested and showed a higher extent of binding than the dominant clone. The  $K_D$ s for the pools were calculated to be approximately 500 nM. The dissociations constants were also calculated as described above. Table 18 shows the rRmY pool clones after Round 6 of selection to h-IgE where the dominant clone was AMX(123).A1 making up 40% of the 96 clones, along with 8 other sequence families.

**[00240]** Table 18. Corresponding cDNAs of the Individual Clone Sequence of rRmY Pool Clones After Round 6 of Selection to h-IgE.

```
SEQ ID No.355   AMX(123).A1
GGGAGAGGAGAGAACGTTCTACGATCTGGGCGAGCCAGTCTGACTGAGGAAGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.356   ARX34P1.B07
GGGAGAGGAGAGAACGTTCTACGAAGAAGATATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.357   ARX34P1.A07
GGGAGAGGAGAGAACGTTCTACGAAAAAGATATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.358   ARX34P1.A01
GGGAGAGGAGAGAACGTTCTACGAAAAAGATATGAGAGAAAGGATTAAGAGGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.359   ARX34P1.G05
GGGAGAGGAGAGAACGTTCTACGAAAAAGACATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.360   ARX34P1.F09
GGGAGAGGAGAGAACGTTCTACNAAAAAGTATATGAGAGAAAGGATTAANAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.361   ARX34P1.B02
GGGAGAGGAGAGAACGTTCTACGAAAAAGATATGAGAGAAAGGATTGAGAGATGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.362   ARX34P1.G02
GGGAGAGGAGAGACGTTCTACGAAAAAGATATGGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.363   ARX34P1.A04
GGGAGAGGAGAGAACGTTCTACGAAAAAGATATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.364   ARX34P1.G06
GGGAGAGGAGAGAACGTTCTACGAAANAAGATACATAGTAGAAAGGATTAATAAGACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.365   ARX34P1.E05
GGGAGAGGAGAGAACGTTCTACAGGCGTGTGGTAGGGTACGACGAGGCATGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.366   ARX34P1.B11
GGGAGAGGAGAGAACGTTCTACGCAAAAATGTGATGCGAGGTAATGGAACGCCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.367   ARX34P1.B01
GGGAGAGGAGAGAACGTTCTACGGACCTCAGCGATAGGGGTTGAAACCGACACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.368   ARX34P1.H06
GGGAGAGGAGAGAACGTTCTACATGGTCGGATGCTGGGGAGTAGGCAAGGTTGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.369   ARX34P1.C12
GGGAGAGGAGAGAACGTTCTACGTATCGGCGGAGCGAAGCATCCGGGAGCGTTGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.370   ARX34P1.C09
GGGAGAGGAGAGAACGTTCTACGTATTGGCGCGCAAGCATCCGGGAGCGTTGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.371   ARX34P1.A11
GGGAGAGGAGAGAACGTTCTACTTATACCTGACGGCCGGAGGCGCATAGGTGCGTGTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.372   ARX34P1.H09
GGGAGAGGAGAGAACGTTCTACATGGTCGGATGCTGGGGAGTAGGCAAGGTTGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.373   ARX34P1.B05
GGGAGAGGAGAGAACGTTCTACACGAGAGTACTGAGGCGCTTGGTACAGAGTGTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.374   ARX34P1.B10
GGGAGAGGAGAGAACGTTCTACAGAAGGTAGAAAAAGGATAGCTGTGAGAAGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.375   ARX34P1.C01
GGGAGAGGAGAGAACGTTCTACTGAGGGATAATACGGGTGGGATTGTCTTCCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.376   ARX34P1.D04
GGGAGAGGAGAGAACGTTCTACATTGAGCGTTGAAGTTGGGGAAGCTCCGAGGCCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.377   ARX34P1.E02
GGGAGAGGAGAGAACGTTCTACGCGGAGATATACAGCGAGGTAATGGAACGCCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.378   ARX34P1.F01
GGGAGAGGAGAGAACGTTCTACGAAGACAGCCCAATAGCGGCACGGAAGTTGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.379   ARX34P1.G03
GGGAGAGGAGAGAACGTTCTACCGGTTGAGGGCTCGCGTGGAAGGGCCAACACGCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.380   ARX34P1.H01
GGGAGAGGAGAGAACGTTCTACATATCAATAGACTCTTGACGTTTGGGTTTGGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.381   ARX34P1.H02
GGGAGAGGAGAGAACGTTCTACAGTGAAGGAAAAGTAAGTGAAGGTGTGCGCTGTCGATCGATCGATCGATGAAGGGCG
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SEQ ID No.382    ARX34P1.H03  
GGGAGAGGAGAGAAACGTTCTACGGATGAAATGAGTGTCTGCGATAGGTTAAGCGCTGTCGATCGATCGATCGATGAAGGGCG  
SEQ ID No.383    ARX34P1.H10  
GGGAGAGGAGAGAAACGTTCTACGGAGGAAATGTGTGTCTGCGATAGGTTAAGCGCTGTCGATCGATCGATCGATGAAGGGCG

### Example 9 rRmY and rGmH 2'-OMe SELEX™ against PDGF-BB

**[00241]** Selections were performed to identify aptamers containing 2'-OMe C, U and 2'-OH G, A (rRmY), and the other 2'-O-Methyl A, C, and U and 2'-OH G (rGmH). All selections were direct selections against human PDGF-BB protein target which had been immobilized on a hydrophobic plate. Selections yielded pools significantly enriched for h\_PDGF-BB binding versus naïve, unselected pool. Individual clone sequences for PDGF-BB are reported herein.

**[00242] Pool Preparation.** A DNA template with the sequence

5'-GGGAGAGGAGAGAACGTTCTACNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNC  
GCTGTCGATCGATCGATCGATG-3' (SEQ ID NO:459) was synthesized using an ABI  
EXPEDITE™ DNA synthesizer, and deprotected by standard methods. The templates were  
amplified with the primers PB.118.95.G 5'-GGGAGAGGAGAGAACGTTCTAC-3' (SEQ ID  
NO:460) and STC.104.102.A 5'-CATCGATCGATCGATCGACAGC-3'(SEQ ID NO:461) and  
then used as a template (200 nm template was used for round 1, and for subsequent rounds  
approximately 50 nM, a 1/10 dilution of an optimized PCR reaction, using conditions described  
herein, was used) for *in vitro* transcription with Y639F single mutant T7 RNA polymerase.  
Transcriptions were done using 200 mM HEPES, 40 mM DTT, 2 mM spermidine, 0.01%  
TritonX-100, 10% PEG-8000, 5 mM MgCl<sub>2</sub>, 1.5 mM MnCl<sub>2</sub>, 500 μM NTPs, 500 μM GMP, 0.01  
units/μl inorganic pyrophosphatase, and Y639F single mutant T7 polymerase. Two different  
compositions were transcribed rRmY and rGmH.

**Selection.** Each round of selection was initiated by immobilizing 20 pmoles of PDGF-BB to the surface Nunc Maxisorp hydrophobic plates for 2 hours at room temperature in 100  $\mu$ L of 1X Dulbecco's PBS. The supernatant was then removed and the wells were washed 4 times with 120  $\mu$ L wash buffer (1X DPBS, 0.2% BSA, and 0.05% Tween-20). Pool RNA was heated to 90  $^{\circ}$ C for 3 minutes and cooled to room temperature for 10 minutes to refold. In round 1, a positive selection step was conducted. Briefly,  $1 \times 10^{14}$  molecules (0.2 nmoles) of pool RNA were incubated in 100  $\mu$ L binding buffer (1X DPBS and 0.05% Tween-20) in the wells with immobilized protein target for 1 hour. The supernatant was then removed and the wells were



washed 4X with 120  $\mu$ L wash buffer. In subsequent rounds a negative selection step was included. The pool RNA was also incubated for 30 minutes at room temperature in empty wells to remove any plastic binding sequences from the pool before the positive selection step. The number of washes was increased after round 4 to increase stringency. In all cases, the pool RNA bound to immobilized PDGF-BB was reverse transcribed directly in the selection plate after by the addition of RT mix (3' primer, STC.104.102.A, and Thermoscript RT, Invitrogen) followed by incubated at 65 °C for 1 hour. The resulting cDNA was used as a template for PCR (Taq polymerase, New England Biolabs) "Hot start" PCR conditions coupled with a 60 °C annealing temperature were used to minimize primer-dimer formation. Amplified pool template DNA was desalted with a Centrisep column according to the manufacturer's recommended conditions and used to program transcription of the pool RNA for the next round of selection. The transcribed pool was gel purified on a 10 % polyacrylamide gel every round.

[00243] Although the naïve pool does bind to PDGF-BB, the rRmY PDGF-BB selection was enriched after 4 rounds over the naïve pool. The selection stringency was increased and the selection was continued for 8 more rounds. At round 12 the pool is enriched over the naïve pool, but the  $K_D$  is very high. The rGmH selection was enriched over the naïve pool binding at round 10. The pool  $K_D$  is also approximately 950 nM or higher. The pools were cloned using TOPO TA cloning kit (Invitrogen) and submitted for sequencing. After 12 rounds of PDGF-BB pool selection clones were transcribed and sequenced. Table 19 shows the clone sequences. Figure 13(A) shows a binding plot of round 12 pools for rRmY pool PDGF-BB selection and Figure 13(B) shows a binding plot of round 10 pools for rGmH pool PDGF-BB selection. Dissociation constants were again measured using the sandwich filter binding technique. Dissociation constants ( $K_{DS}$ ) were estimated fitting the data to the equation: fraction RNA bound = amplitude\* $K_D/(K_D + [PDGF-BB])$ .

[00244] Table 19. Corresponding cDNAs of the Individual Clone Sequence of rRmY Pool Clones After Round 12 of Selection to PDGF-BB.

```
SEQ ID No.384 PDGF-BB ARX36.SCK.E05
GGGAGAGGAGAGAACGTTCTACATCCTTGCGTATGATCGGCATCGTAAGACACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.385 PDGF-BB ARX36.SCK.F05
GGGAGAGGAGAGAACGTTCTACATCCTTGCGTATGATCGGCATCGTAAGACACGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.386 PDGF-BB ARX36.SCK.E01
GGGAGAGGAGAGAACGTTCTACGATCGAAGTCGTGACAGAAACCACTCGCTGTCGATCGATCGATCGATGAAGGGCG
SEQ ID No.387 PDGF-BB ARX36.SCK.F01
GGGAGAGGAGAGAACGTTCTACGATCGAAGTCGTGACAGAAACCACTCGCTGTCGATCGATCGATCGATGAAGGGCG
```

SEQ ID No.388 PDGF-BB ARX36.SCK.G01  
 GGGAGAGGAGAGAACGTTCTACGGAAGGTTGGCGAAACGAAGAAGAAATTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.389 PDGF-BB ARX36.SCK.G02  
 GGGAGAGGAGAGAACGTTCTACGGAAGGTTGGCGAAACGAAGAANAATTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.390 PDGF-BB ARX36.SCK.F04  
 GGGAGAGGAGAGAACGTTCTACTGGGAGTTGCGGTGTTTTGCGGTGGATTGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.391 PDGF-BB ARX36.SCK.E04  
 GGGAGAGGAGAGAACGTTCTACTGGGAGTTGCGGTGTTTTGCGGTGGATTGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.392 PDGF-BB ARX36.SCK.F02  
 GGGAGAGGAGAGAACGTTCTACAAGATTGTAGATCAACAGCGAAGGCGTGGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.393 PDGF-BB ARX36.SCK.E02  
 GGGAGAGGAGAGAACGTTCTACAAGATTGTAGATCAACAGCGAAGGCGTGGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.394 PDGF-BB ARX36.SCK.A02  
 GGGAGAGGAGAGAACGTTCTACAAANAAGATNNCCANCNNGAGANAAGGAGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.395 PDGF-BB ARX36.SCK.A03  
 GGGAGAGGAGAGAACGTTCTACAAACATCGAAGATCGAACTGAAAAGGAGGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.396 PDGF-BB ARX36.SCK.A06  
 GGGAGAGGAGAGAACGTTCTCATGTGCATGCAAGGTGGGGCTGACACGAGCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.397 PDGF-BB ARX36.SCK.B01  
 GGGAGAGGAGAGAACGTTCTACAAGGAGTAGATCGACAGAATAGAAAAATCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.398 PDGF-BB ARX36.SCK.B02  
 GGGAGAGGAGAGAACGTTCTACAAAAGGTAAAGGTCAAAAAGCGCAACGTTGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.399 PDGF-BB ARX36.SCK.D04  
 GGGAGAGGAGAGAACGTTCTACAAAAGGAGGCGAAATTAAGTGAGACAATGTGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.400 PDGF-BB ARX36.SCK.B04  
 GGGAGAGGAGAGAACGTTCTACAAAATCCACAAACATAGCTGTAATTGCTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.401 PDGF-BB ARX36.SCK.B05  
 GGGAGAGGAGAGAACGTTCTACAAGAACATATAACATTTTGGTTGAGAGCAACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.402 PDGF-BB ARX36.SCK.D03  
 GGGAGAGGAGAGAACGTTCTACAAGAGTCNACGATTTTCNATCACAATGTGGCTGTCGTCNATCGATCGATCGATGAAGGGCG  
 SEQ ID No.403 PDGF-BB ARX36.SCK.C01  
 GGGAGAGGAGAGAACGTTCTACAAGCAAGCAAAAAAGTATCGACAGAAGTGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.404 PDGF-BB ARX36.SCK.D06  
 GGGAGAGGAGAGAACGTTCTACAAGTAATATCAGAGCAATCGGAATAAGAGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.405 PDGF-BB ARX36.SCK.D02  
 GGGAGAGGAGAGAACGTTCTACAGACTTCGATGCGATGGATTGGAATGTGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.406 PDGF-BB ARX36.SCK.C03  
 GGGAGAGGAGAGAACGTTCTACAGAAAGATTACAGGAACAAATACACGTGCGGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.407 PDGF-BB ARX36.SCK.F06  
 GGGAGAGGAGAGAACGTTCTACAGAAATCAATCGAGGTGATCGTTATATAGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.408 PDGF-BB ARX36.SCK.C04  
 GGGAGAGGAGAGAACGTTCTACAGATTGGATCGACAATCTCGTAGAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.409 PDGF-BB ARX36.SCK.C06  
 GGGAGAGGAGAGAACGTTCTACAATGCAAGTTTAAGTGTGGTGTCAAACGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.410 PDGF-BB ARX36.SCK.G03  
 GGGAGAGGAGAGAACGTTCTACAAATAAAGACACGAAGATCGACGGAGACTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.411 PDGF-BB ARX36.SCK.F03  
 GGGAGAGGAGAGAACGTTCTACGAAGATGTGTTAAGAATCGAGGTTTTCGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.412 PDGF-BB ARX36.SCK.C02  
 GGGAGAGGAGAGAACGTTCTACGAGTTGGCACGCATGTATAGGTATTTTGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.413 PDGF-BB ARX36.SCK.B03  
 GGGAGAGGAGAGAACGTTCTACGAAAAAGAGATGAGAGAAAGGATTAAGAGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.414 PDGF-BB ARX36.SCK.B06  
 GGGAGAGGAGAGAACGTTCTACGAAAAGGAAAAAACGATCGGCAGAGTCCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.415 PDGF-BB ARX36.SCK.C05  
 GGGAGAGGAGAGAACGTTCTACGATTAAGGAACATTTACGCGAATACATGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.416 PDGF-BB ARX36.SCK.D01  
 GGGAGAGGAGAGAACGTTCTACGACGTTTGTCTGAAAAATAGGACAGAAGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.417 PDGF-BB ARX36.SCK.E03  
 GGGAGAGGAGAGAACGTTCTACGAAGATGTGTTAAGAAATCGAGGTTTTTCGACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.418 PDGF-BB ARX36.SCK.A04  
 GGGAGAGGAGAGAACGTTCTACCGAGATCGAAAGGTAAGAGAAAATTCATGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.419 PDGF-BB ARX36.SCK.A05  
 GGGAGAGGAGAGAACGTTCTACTAAGATTCTGTCGTTCCAGACAGAGAAAGCGACGCTGTCGATCGATCGATCGATGAAGGGCG

[00245] Table 20. Corresponding cDNAs of the Individual Clone Sequence of rGmH Pool  
 Clones After Round 10 of Selection to PDGF-BB.

SEQ ID No.420 PDGF-BB ARX36.SCK.E08.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTGGCGACGATCTGTGACCTGAATTTTGTCCGCTGTCGATCGATCGATCGATGAAGGGCG

SEQ ID No.421 PDGF-BB ARX36.SCK.F08.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTGGCGACGATCTGTGACCTGAATTTTGTCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.422 PDGF-BB ARX36.SCK.E09.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTGGTCTCAGCAGCTTTTAACAAAGTATCCCCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.423 PDGF-BB ARX36.SCK.F09.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTGGTCTCAGCAGCTTTTAACAAAGTATCCCCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.424 PDGF-BB ARX36.SCK.F07.M13F  
 GGGAGAGGAGAGAACGTTCTACCGCTATTTGTTTCATTGAAGGACTTGTACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.425 PDGF-BB ARX36.SCK.E07.M13F  
 GGGAGAGGAGAGAACGTTCTACCGCTATTTGTTTCATTGAAGGACTTGTACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.426 PDGF-BB ARX36.SCK.E11.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTATTGAGGTTGATTGGAAGTGCTATGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.427 PDGF-BB ARX36.SCK.F11.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTATTGAGGTTGATTGGAAGTGCTATGTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.428 PDGF-BB ARX36.SCK.F10.M13F  
 GGGAGAGGAGAGAACGTTCTACTGAAGATGTTATGATGATTGACGAGGAGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.429 PDGF-BB ARX36.SCK.E10.M13F  
 GGGAGAGGAGAGAACGTTCTACTGAAGATGTTATGATGATTGACGAGGAGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.430 PDGF-BB ARX36.SCK.E12.M13F  
 GGGAGAGGAGAGAACGTTCTACTGTCTGAGTGTGCGCGCTTGTGTGATGTTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.431 PDGF-BB ARX36.SCK.F12.M13F  
 GGGAGAGGAGAGAACGTTCTACTGTCTGAGTGTGCGCGCTTGTGTGATGTTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.432 PDGF-BB ARX36.SCK.A07.M13F  
 GGGAGAGGAGAGAACGTTCTACGTGATGGCTGTGAATGAGTAGTTCGAATACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.433 PDGF-BB ARX36.SCK.C12.M13F  
 GGGAGAGGAGAGAACGTTCTACGTGAAATCAAGTTGTTAATTTGGGGAATCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.434 PDGF-BB ARX36.SCK.B07.M13F  
 GGGAGAGGAGAGAACGTTCTACGTATAAGGCCGTAAACGGGTAGCGAGTGGTTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.435 PDGF-BB ARX36.SCK.A09.M13F  
 GGGAGAGGAGAGAACGTTNTACGTGGGCGAAGGAGCTGCGGGCGTTGNAGTTTGTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.436 PDGF-BB ARX36.SCK.A11.M13F  
 GGGAGAGGAGAGAACGTTCTACGTATCCTAGTCTGAGATCGGATTTTCTTGGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.437 PDGF-BB ARX36.SCK.C09.M13F  
 GGGAGAGGAGAGAACGTTCTACGTTTGGCAGTGTGGTTCGACGCTGAATGCGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.438 PDGF-BB ARX36.SCK.A08.M13F  
 GGGAGAGGAGAGAACGTTCTACGGATTGATAGGGATTGAGATGAGGTCTTGTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.439 PDGF-BB ARX36.SCK.D07.M13F  
 GGGAGAGGAGAGAACGTTCTACGATGTCGTTAGATTACTTATTGCTATCTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.440 PDGF-BB ARX36.SCK.D08.M13F  
 GGGAGAGGAGAGAACGTTCTACGATGCCTGGCGGAAACGGAGCCTGGGATTTGCTGTCNATCGATCGATCGATGAAGGGCG  
 SEQ ID No.441 PDGF-BB ARX36.SCK.B11.M13F  
 GGGAGAGGAGAGAACGTTCTACGAGGATTGACGTGTGTGCTAGAGTACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.442 PDGF-BB ARX36.SCK.D09.M13F  
 GGGAGAGGAGAGAACGTTCTACGATATTATGCGTCCCTTGAGGATACACGGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.443 PDGF-BB ARX36.SCK.B10.M13F  
 GGGAGAGGAGAGAACGTTCTACAGGATAACTGTAGCGATAAGTAACGATGTCGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.444 PDGF-BB ARX36.SCK.C10.M13F  
 GGGAGAGGAGAGAACGTTCTACAAGAAGTGTGGCCGAGAGACGAAATGCACGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.445 PDGF-BB ARX36.SCK.A10.M13F  
 GGGAGAGGAGAGAACGTTCTACCATATCTTCTTCTTTATTCGGTTAGTTGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.446 PDGF-BB ARX36.SCK.B09.M13F  
 GGGAGAGGAGAGAACGTTCTACCTGTGTTGATGCTTCCGTTTGAGATTGCCCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.447 PDGF-BB ARX36.SCK.B12.M13F  
 GGGAGAGGAGAGAACGTTCTACNGTAAGANAANCTATTTAGCCCTTGNNCTGCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.448 PDGF-BB ARX36.SCK.C08.M13F  
 GGGAGAGGAGAGAACGTTCTACCTTGTCTCCTCAATCCTCTTTTGACTCTTGCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.449 PDGF-BB ARX36.SCK.D12.M13F  
 GGGAGAGGAGAGAACGTTCTACCTGATTTTGTCACTGGATTCCGATGGCTTTCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.450 PDGF-BB ARX36.SCK.C11.M13F  
 GGGAGAGGAGAGAACGTTCTACTGTAATAAGGGATGCGTCAGGAACCTGTGTTGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.451 PDGF-BB ARX36.SCK.D11.M13F  
 GGGAGAGGAGAGAACGTTCTACTGCTTCCGGGAATTTGTTTGTGTTTCCGCTGTCGATCGATCGATCGATGAAGGGCG  
 SEQ ID No.452 PDGF-BB ARX36.SCK.C07.M13F  
 GGGAGAGGAGAGAACGTTCTACTTCGTCGGTTGACTTTTCTTCGTGTAGTGTGCTGTCGATCGATTGATCGATGAAGGGCG  
 SEQ ID No.189 PDGF-BB ARX36.SCK.A12.M13F  
 GGGAGAGGAGAGAACGTTCTACTATGAAGGGTTTTAAAGATGACACATTAGCCGCTGTCGATCGATCGATCGATGAAGGGCG

[00246] The present invention having been described by detailed description and the foregoing non-limiting examples, is now defined by the spirit and scope of the following claims.